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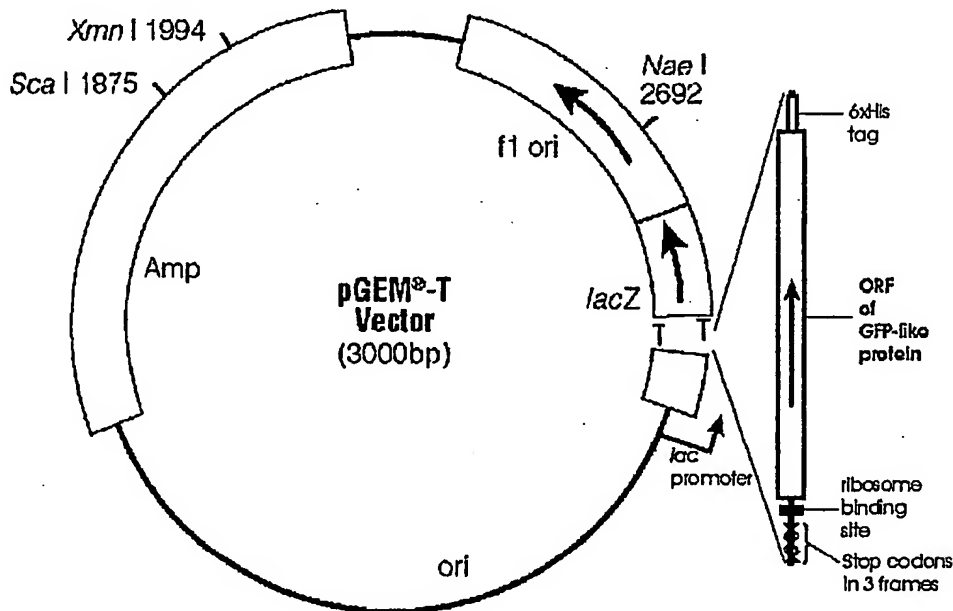
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(54) Title: NOVEL FLUORESCENT AND COLORED PROTEINS, AND POLYNUCLEOTIDES THAT ENCODE THESE PRO-  
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(57) Abstract: The subject invention provides new fluorescent and/or colored proteins, and polynucleotide sequences that encode these proteins. The subject invention further provides materials and methods useful for expressing these detectable proteins in biological systems.

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DESCRIPTIONNOVEL FLUORESCENT AND COLORED PROTEINS, AND  
POLYNUCLEOTIDES THAT ENCODE THESE PROTEINS

5

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10

Cross-Reference to a Related Application

This application claims the benefit of U.S. provisional patent application Serial No. 60/472,196, filed May 20, 2003.

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Field of the Invention

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The present invention relates to novel fluorescent and colored proteins, and their use. These materials and methods are particularly advantageous for labeling and detection technology. Specifically, exemplified are novel colored and/or fluorescent proteins, and mutants thereof, isolated from marine organisms. These new proteins offer a wider array of colors and biochemical features compared to existing wild-type green fluorescent protein (GFP) or its modified variants utilized in current labeling and detection technology.

25

Background of the Invention

30

Genetic markers are important for monitoring gene expression and tracking movement of proteins in cells. Markers have been extensively used for monitoring biological activity of genetic elements such as promoters, enhancers and terminators, and other aspects of gene regulation in numerous biological systems. Over the years numerous marker genes have been developed and utilized widely in molecular and genetic studies aimed at the identification, isolation and characterization of genetic regulatory elements and genes, and the development of gene transfer techniques.

In general, markers can be grouped into selectable markers and reporter markers. Selectable markers are typically enzymes with catalytic capability to

convert chemical substrates usually harmful to host cells into non-toxic products, thus providing transformed host cells a conditionally selectable growth advantage under selective environment and allowing the recovery of stable transformants after transformation. A number of commonly used selectable markers include those that confer resistance characteristics to antibiotics (Gritz and Davies 1983; Bevan *et al.*, 1983) and herbicides (De Block *et al.*, 1987), and those with enzymatic activity to detoxify metabolic compounds that can adversely affect cell growth (Joersbo and Okkels 1996).

Reporter markers are compounds that provide biochemically assayable or identifiable activities. Reporter markers have been widely used in studies to reveal biological functions and modes of action of genetic elements such as promoters, enhancers, terminators, and regulatory proteins including signal peptides, transcription factors and related gene products. Over the years, several reporter markers have been developed for use in both prokaryotic and eukaryotic systems, including  $\beta$ -galactosidase (LacZ) (Stanley and Luzio 1984),  $\beta$ -glucuronidase (GUS) (Jefferson *et al.*, 1987; U.S. Patent No. 5,268,463), chloramphenicol acetyltransferase (CAT) (Gorman *et al.*, 1982), green fluorescent protein (GFP) (Prasher *et al.*, 1992; U.S. Patent No. 5,491,084) and luciferase (Luc) (Ow *et al.*, 1986).

Among reporter markers, GUS offers a sensitive and versatile reporting capability for gene expression in plants.  $\beta$ -glucuronidase or GUS, encoded by the *uidA* gene from *Escherichia coli*, catalyzes the conversion of several colorigenic and fluorogenic glucorogenic substrates such as p-nitrophenyl  $\beta$ -D-glucuronide and 4-methylumbelliferyl  $\beta$ -D-glucuronide into easily detectable products. GUS activity can be measured by highly sensitive colorimetric and fluorimetric methods (Jefferson *et al.*, 1987). However, the GUS assay often requires total destruction of the sample tissues or exposure of sample tissues to phytotoxic chemical substrates. This prevents repeated use of the same sample tissue for continuous expression analysis and precludes the recovery of transformants from analyzed materials.

Recently, GFP isolated from the Pacific Northwest jellyfish (*Aequorea victoria*) has become an important reporter marker for non-destructive analysis of gene expression. GFP fluoresces *in vivo* by receiving light energy without the involvement of any chemical substrates. Thus, GFP is especially suitable for real



time and continuous monitoring of temporal and spatial control of gene expression and protein activities without any physical damage to assay samples.

The gene for GFP has been cloned and used as a reporter gene, which can be expressed as a functional transgene in living organisms, marking the organisms with fluorescent color and thus allowing detection of those organisms. Accordingly, GFP has become a versatile fluorescent marker for monitoring a variety of physiological processes, visualizing protein localization and detecting the expression of transferred genes in various living systems, including bacteria, fungi, and mammalian tissues.

This *in vivo* labeling and detection technology was originally based on a single fluorescent protein: the green fluorescent protein from *Aequorea victoria*. Numerous modifications have been made to alter the spectral properties of GFP to provide for significant enhancement in fluorescence intensity (Prasher *et al.*, 1992; Cubitt *et al.*, 1995, Heim *et al.*, 1994, 1995; Cormack *et al.*, 1996; U.S. Patent No. 5,804,387). In addition, GFP genes have been modified to contain more silent base mutations that correspond to codon-usage preferences in order to improve its expression efficacy, making it a reporter gene in both animal and plant systems (U.S. Patent Nos. 5,874,304; 5,968,750; and 6,020,192).

In addition to GFP, there are now a number of other fluorescent proteins, substantially different from GFP, which are being developed into biotechnology tools. Most prominent of these proteins is the red fluorescent protein DsRed. See, for example, Labas, Y. A., N. G. Gurskaya, Y. G. Yanushevich, A. F. Fradkov, K. A. Lukyanov, S. A. Lukyanov and M. V. Matz. (2002) "Diversity and evolution of the green fluorescent protein family" *Proc Natl Acad Sci USA* 99:4256-4261 and Matz, M. V., K. A. Lukyanov and S. A. Lukyanov (2002) "Family of the green fluorescent protein: journey to the end of the rainbow" *Bioessays* 24: 953-959.

Labeling technologies based on GFP and related proteins have become indispensable in such areas as basic biomedical research, cell and molecular biology, transgenic research and drug discovery. The number of PubMed records containing the phrase "green fluorescent protein" exceeds 5500 only within the last three years. Demand for labeling and detection based on the fluorescent protein technology is large and steady.

Currently, there are very few known natural pigments essentially encoded by a single gene, wherein both the substrate for pigment biosynthesis and the necessary

catalytic moieties are provided within a single polypeptide chain. The limited availability of fluorescent marker proteins makes the current technology based on fluorescent proteins very expensive, rendering it unaffordable and inaccessible to many mid-size (or smaller) companies that are interested in using the technology. Therefore, there is a need for less expensive, readily available fluorescent and/or colored materials.

### Brief Summary of the Invention

The subject invention provides new fluorescent and/or colored proteins, and polynucleotide sequences that encode these proteins. The subject invention further provides materials and methods useful for expressing these detectable proteins in biological systems.

In specific embodiments, the subject invention provides a red fluorescent protein from *Scolymia cubensis* scubRFP, featuring rapid conversion from immature green to mature red form under UV-A light; and three fluorescent proteins from *Montastraea cavernosa*, namely g5.2 (cyan), mc6 (green) and R7 (green) proteins. The invention also includes proteins substantially similar to, or mutants or variants of, the exemplified proteins.

Another aspect of the subject invention pertains to polynucleotide sequences that encode the detectable proteins of the present invention. In one embodiment, the present invention provides polynucleotide constructs comprising cDNA encoding novel colored and/or fluorescent proteins and mutants thereof.

The subject invention also provides proteins from *Acropora* ("staghorn corals") and *Agarica fragilis* ("fragile saucer coral"), as well as polynucleotides encoding these proteins.

In one embodiment, the invention provides nucleotide sequences of the inserts in pGEM-T vector (Promega), the conceptual translations of these inserts, and special properties of purified protein products.

The proteins and polynucleotides of the present invention can be used as described herein as colored and/or fluorescent (detectable) labels in a variety of ways, including but not limited to, as reporter genes for monitoring gene expression in living organisms, as protein tags for tracing the location of proteins within living cells and organisms, as reporter molecules for engineering various protein-based biosensors,

and as genetically encoded pigments for modifying color and/or fluorescence of living organisms or their parts.

In a specific embodiment, the proteins of the subject invention can be used in molecular fluorescent tagging whereby the coding region of a protein of interest is fused with the coding region for a fluorescent protein of the subject invention. The product of such a gene shows the functional characteristics of the protein of interest, but bears the fluorescent label allowing tracing its movements.

Advantageously, the present invention provides proteins and polynucleotides to improve on the current technology of labeling and detection by offering a wider choice of colors and biochemical features never before provided by GFP and its modified variants.

#### Brief Description of the Figures

Figure 1 shows design of bacterial expression constructs for the proteins of interests of the present invention.

Figure 2 shows the bacterial colonies expressing genes described in the present invention (cyan, green and red) under UV-A light. The bacterial colonies affected by the expression show red and greenish color and fluorescent appearance. These bacterial colonies are normally non-fluorescent under UV-A light.

Figure 3A-3B shows maturation of scubRFP under low-intensity UV-A light, resulting in conversion from a green-emitting form (emission maximum 520 nm) into red-emitting form (emission maximum 575 nm). Figure 3A is a graph showing the change in ratio or emission amplitudes of 520 and 575 nm. Figure 3B graph shows changes in the emission spectra.

Figure 4 shows the excitation and emission spectra of *A. aculeus* 1-1 (green).

Figure 5 shows the excitation and emission spectra of *A. aculeus* 1-2 (green).

Figure 6 shows the excitation and emission spectra of *A. aculeus* 2-1 (green).

Figure 7 shows the excitation and emission spectra of *A. aculeus* 2-2 (green).

Figure 8 shows the excitation and emission spectra of *A. aculeus* 3-1 (green).

Figure 9 shows the excitation and emission spectra of *A. millepora* 8-2 (cyan).

Figure 10 shows the excitation and emission spectra of *A. millepora* 9-1 (green).

Figure 11 shows the excitation and emission spectra of *A. millepora* 9-2 (green).

Figure 12 shows the excitation and emission spectra of *A. millepora* 10-1 (green).

5 Figure 13 shows the excitation and emission spectra of *A. millepora* 10-2 (cyan).

Figure 14 shows the excitation and emission spectra of *A. millepora* 11-1 (green).

10 Figure 15 shows the excitation and emission spectra of *A. millepora* 12-1 (red).

Figure 16 shows the excitation and emission spectra of *A. nobilis* 15-1 (cyan).

Figure 17 shows the excitation and emission spectra of *A. nobilis* 16-1 (cyan).

Figure 18 shows the excitation and emission spectra of *A. nobilis* 17-1 (green).

15 Figure 19 shows the excitation and emission spectra of *Agaricia fragilis* 1 (green).

Figure 20 shows the excitation and emission spectra of *Agaricia fragilis* 2 (green).

20 Figure 21 shows the excitation and emission spectra of *Agaricia fragilis* 3 (green).

Figure 22 shows the excitation and emission spectra of *Agaricia fragilis* 4 (cyan).

Figure 23 shows the excitation and emission spectra of *Agaricia fragilis* 5 (green).

25 Figure 24 shows the excitation and emission spectra of *Agaricia fragilis* 6 (green).

Figure 25 shows the excitation and emission spectra of *Agaricia fragilis* 8 (cyan).

30 Figure 26 shows the excitation and emission spectra of *A. aculeus* 5-2 (chromoprotein).

Figure 27 shows the excitation and emission spectra of *A. aculeus* 6-1 (chromoprotein).

Figure 28 shows the excitation and emission spectra of *A. hyacinthus* 7-1 (chromoprotein).

Figure 29 shows the excitation and emission spectra of *A. millepora* 14-1 (chromoprotein).

5

#### Brief Description of the Sequences

SEQ ID NO:1 is the 5' heel of an upstream primer used according to the subject invention.

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SEQ ID NO:2 is the 5' heel of a downstream primer used according to the subject invention.

SEQ ID NO:3 is the open reading frame of the cDNA encoding the g5.2 (cyan) protein of interest from *Montastraea cavernosa*.

SEQ ID NO:4 is the open reading frame of the cDNA encoding the mc6 (green) protein of interest from *Montastraea cavernosa*.

15

SEQ ID NO:5 is the open reading frame of the cDNA encoding the R7 (green) protein of interest from *Montastraea cavernosa*.

SEQ ID NO:6 is the open reading frame of the cDNA encoding the scubRFP protein of interest from *Scolymia cubensis*.

SEQ ID NO:7 is the amino acid sequence encoded by SEQ ID NO:3.

20

SEQ ID NO:8 is the amino acid sequence encoded by SEQ ID NO:4.

SEQ ID NO:9 is the amino acid sequence encoded by SEQ ID NO:5.

SEQ ID NO:10 is the amino acid sequence encoded by SEQ ID NO:6.

SEQ ID NO:11 is the bacterial expression construct for the g5.2 (cyan) protein of interest from *Montastraea cavernosa*.

25

SEQ ID NO:12 is the bacterial expression construct for the mc6 (green) protein of interest from *Montastraea cavernosa*.

SEQ ID NO:13 is the bacterial expression construct for the R7 (green) protein of interest from *Montastraea cavernosa*.

30

SEQ ID NO:14 is the bacterial expression construct for the scubRFP protein of interest from *Scolymia cubensis*.

SEQ ID NO:15 is the amino acid sequence encoded by SEQ ID NO:11.

SEQ ID NO:16 is the amino acid sequence encoded by SEQ ID NO:12.

SEQ ID NO:17 is the amino acid sequence encoded by SEQ ID NO:13.

SEQ ID NO:18 is the amino acid sequence encoded by SEQ ID NO:14.

SEQ ID NO:19 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 1-1 in pGEM-T).

5 SEQ ID NO:20 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 1-2 in pGEM-T).

SEQ ID NO:21 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 2-1 in pGEM-T).

SEQ ID NO:22 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 2-2 in pGEM-T).

10 SEQ ID NO:23 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 3-1 in pGEM-T).

SEQ ID NO:24 is the nucleotide sequence insert of the subject invention (Acropora aculeus 5-2 in pGEM-T).

15 SEQ ID NO:25 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 6-1 in pGEM-T).

SEQ ID NO:26 is the nucleotide sequence insert of the subject invention (Acropora hyacinthus 7-1 in pGEM-T).

SEQ ID NO:27 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention Acropora millepora 8-2 in pGEM-T).

20 SEQ ID NO:28 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 9-1 in pGEM-T).

SEQ ID NO:29 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 9-2 in pGEM-T).

25 SEQ ID NO:30 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 10-1 in pGEM-T).

SEQ ID NO:31 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 10-2 in pGEM-T).

SEQ ID NO:32 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 11-1 in pGEM-T).

30 SEQ ID NO:33 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 12-1 in pGEM-T).

SEQ ID NO:34 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 14-1 in pGEM-T).

SEQ ID NO:35 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora nobilis 15-1 in pGEM-T).

SEQ ID NO:36 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora nobilis 16-1 in pGEM-T).

5 SEQ ID NO:37 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora nobilis 17-1 in pGEM-T).

SEQ ID NO:38 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 1 in pGEM-T).

10 SEQ ID NO:39 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 2 in pGEM-T).

SEQ ID NO:40 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 3 in pGEM-T).

SEQ ID NO:41 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 4 in pGEM-T).

15 SEQ ID NO:42 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 5 in pGEM-T).

SEQ ID NO:43 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 6 in pGEM-T).

20 SEQ ID NO:44 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 8 in pGEM-T).

SEQ ID NO:45 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 1-1 in pGEM-T.

SEQ ID NO:46 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 1-2 in pGEM-T.

25 SEQ ID NO:47 is the amino acid sequence of a protein of the subject invention as expressed by the following construct: Acropora aculeus 2-1 in pGEM-T.

SEQ ID NO:48 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 2-2 in pGEM-T.

30 SEQ ID NO:49 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 3-1 in pGEM-T.

SEQ ID NO:50 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 5-2 in pGEM-T.

SEQ ID NO:51 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora aculeus* 6-1 in pGEM-T.

SEQ ID NO:52 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora hyacinthus* 7-1 in pGEM-T.

5 SEQ ID NO:53 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 8-2 in pGEM-T.

SEQ ID NO:54 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 9-1 in pGEM-T.

10 SEQ ID NO:55 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 9-2 in pGEM-T.

SEQ ID NO:56 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 10-1 in pGEM-T.

SEQ ID NO:57 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 10-2 in pGEM-T.

15 SEQ ID NO:58 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 11-1 in pGEM-T.

SEQ ID NO:59 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 12-1 in pGEM-T.

20 SEQ ID NO:60 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora millepora* 14-1 in pGEM-T.

SEQ ID NO:61 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora nobilis* 15-1 in pGEM-T.

SEQ ID NO:62 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora nobilis* 16-1 in pGEM-T.

25 SEQ ID NO:63 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Acropora nobilis* 17-1 in pGEM-T.

SEQ ID NO:64 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Agaricia fragilis* 1 in pGEM-T.

30 SEQ ID NO:65 is the amino acid sequence of a protein of the subject invention as expressed by the following construct: *Agaricia fragilis* 2 in pGEM-T.

SEQ ID NO:66 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Agaricia fragilis* 3 in pGEM-T.



SEQ ID NO:67 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Agaricia fragilis* 4 in pGEM-T.

SEQ ID NO:68 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Agaricia fragilis* 5 in pGEM-T.

5 SEQ ID NO:69 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Agaricia fragilis* 6 in pGEM-T.

SEQ ID NO:70 is the amino acid sequence of a protein of the subject invention as encoded by the following construct: *Agaricia fragilis* 8 in pGEM-T.

SEQ ID NO:71 is the coding region of the construct of SEQ ID NO:45.

10 SEQ ID NO:72 is the coding region of the construct of SEQ ID NO:46.

SEQ ID NO:73 is the coding region of the construct of SEQ ID NO:47.

SEQ ID NO:74 is the coding region of the construct of SEQ ID NO:48.

SEQ ID NO:75 is the coding region of the construct of SEQ ID NO:49.

SEQ ID NO:76 is the coding region of the construct of SEQ ID NO:50.

15 SEQ ID NO:77 is the coding region of the construct of SEQ ID NO:51.

SEQ ID NO:78 is the coding region of the construct of SEQ ID NO:52.

SEQ ID NO:79 is the coding region of the construct of SEQ ID NO:53.

SEQ ID NO:80 is the coding region of the construct of SEQ ID NO:54.

SEQ ID NO:81 is the coding region of the construct of SEQ ID NO:55.

20 SEQ ID NO:82 is the coding region of the construct of SEQ ID NO:56.

SEQ ID NO:83 is the coding region of the construct of SEQ ID NO:57.

SEQ ID NO:84 is the coding region of the construct of SEQ ID NO:58.

SEQ ID NO:85 is the coding region of the construct of SEQ ID NO:59.

SEQ ID NO:86 is the coding region of the construct of SEQ ID NO:60.

25 SEQ ID NO:87 is the coding region of the construct of SEQ ID NO:61.

SEQ ID NO:88 is the coding region of the construct of SEQ ID NO:62.

SEQ ID NO:89 is the coding region of the construct of SEQ ID NO:63.

SEQ ID NO:90 is the coding region of the construct of SEQ ID NO:64.

SEQ ID NO:91 is the coding region of the construct of SEQ ID NO:65.

30 SEQ ID NO:92 is the coding region of the construct of SEQ ID NO:66.

SEQ ID NO:93 is the coding region of the construct of SEQ ID NO:67.

SEQ ID NO:94 is the coding region of the construct of SEQ ID NO:68.

SEQ ID NO:95 is the coding region of the construct of SEQ ID NO:69.

SEQ ID NO:96 is the coding region of the construct of SEQ ID NO:70.

### Detailed Description of the Invention

5 The present invention provides novel fluorescent and colored proteins isolated from marine organisms other than *Aequorea Victoria*. In a particularly preferred embodiment, these proteins are red fluorescent proteins featuring rapid conversion from immature green to mature red under UV-A light. Specifically exemplified herein are scubRFP from *Scolymia cubensis*; and g5.2 (cyan), mc6 (green) and R7 (green) proteins, from *Montastraea cavernosa*.

10 The subject invention further provides polynucleotide sequences encoding these proteins. These polynucleotide sequences include open reading frames encoding the specific exemplified detectable proteins, as well as expression constructs for expressing these proteins, for example, in bacterial hosts.

15 The proteins of the present invention can be readily, expressed by any one of the recombinant technology methods known to those skilled in the art having the benefit of the instant disclosure. The preferred method will vary depending upon many factors and considerations, including the host, and the cost and availability of materials and other economic considerations. The optimum production procedure for a given situation will be apparent to those skilled in the art having the benefit of the  
20 current disclosure.

The subject invention also concerns cells transformed with a polynucleotide of the present invention comprising a nucleotide sequences encoding a novel detectable protein. These cells may be prokaryotic or eukaryotic, plant or animal. In one embodiment, animals, such as fish, are transformed to provide them with a unique  
25 color or ability to fluoresce. Polynucleotides providing the markers of the present invention are stable in a diverse range of hosts, including prokaryotic and eukaryotic organisms, and the translation products are fully functional and capable of providing assayable characteristics.

30 In another embodiment, the present invention provides methods to synthesize colored and fluorescent proteins in a recombinant cell.

In a specific embodiment, the proteins of the subject invention can be used in molecular fluorescent tagging whereby the coding region of a protein of interest is fused with the coding region for a fluorescent protein of the subject invention. The

product of such a gene shows the functional characteristics of the protein of interest, but bears the fluorescent label allowing tracing its movements. See, for example, Eichinger, L., S. S. Lee and M. Schleicher (1999) "Dictyostelium as model system for studies of the actin cytoskeleton by molecular genetics" *Microsc Res Tech* 47:124-134; Falk, M. M. and U. Lauf (2001) "High resolution, fluorescence deconvolution microscopy and tagging with the autofluorescent tracers CFP, GFP, and YFP to study the structural composition of gap junctions in living cells" *Microsc Res Tech* 52:251-262; Kallal, L. and J. L. Benovic (2000) "Using green fluorescent proteins to study G-protein-coupled receptor localization and trafficking" *Trends Pharmacol Sci* 21:175-180; and Laird, D. W., K. Jordan, T. Thomas, H. Qin, P. Fistouris and Q. Shao (2001) "Comparative analysis and application of fluorescent protein-tagged connexins" *Microsc Res Tech* 52:263-272.

In a further embodiment, the subject invention concerns polynucleotides comprising an in-frame fusion of nucleotide sequences encoding multiple genetic markers. In one embodiment, the polynucleotides encode the genetic markers GUS, and a detectable protein of the subject invention.

The subject invention helps to provide a more abundant and diverse collection of proteins, which can be used in place of a GFP protein, such that new proteins are readily available for commercial exploitation by small companies that cannot take advantage of the current technology for financial reasons.

### Definitions

As used herein, the terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide, ribonucleotide, or a mixed deoxyribonucleotide and ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides.

As used herein, "a vector" is a DNA sequence having the elements necessary for the transcription/translation of a gene. Such elements would include, for example, promoters. Various classes of promoters are well known in the art and can be obtained commercially or assembled from the sequences and methods, which are also well known in the art. A number of vectors are available for expression and/or

cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).

As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

#### Detectable Proteins

The subject invention provides novel fluorescent and/or colored proteins. These proteins are exemplified by scubRFP from *Scolymia cubensis* (SEQ ID NO. 7); and g5.2 (cyan) (SEQ ID NO. 8), mc6 (green) (SEQ ID NO. 9) and R7 (green) (SEQ ID NO. 10) proteins, from *Montastraea cavernosa*.

The novel colored and fluorescent proteins of the present invention can be detected using standard long-wave UV light sources or, preferably, optical designs appropriate for detecting agents with the excitation/emission characteristics of the proteins exemplified herein (see, for example, Figures 2-29). These proteins are referred to herein as "detectable proteins" or "marker proteins." The interaction of two or more residues of the protein and external agents such as molecular oxygen give rise to the colored and/or fluorescent feature of the proteins.

Advantageously, the use of these proteins facilitate real-time detection *in vivo*, a substrate is not required, and the relatively small size make the proteins very advantageous.

Substitution of amino acids other than those specifically exemplified or naturally present in the genetic marker proteins of the invention are also contemplated within the scope of the present invention. Such substitutions will create "variant proteins" within the scope of the subject invention. Variants and fragments preferably have emission and excitation maxima within 10 nm of the values shown in Figures 2-29. For example, non-natural amino acids can be substituted for the amino acids of the marker proteins, so long as a marker protein having the substituted amino acids retains its ability to be detected through fluorescence and/or color. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline,

hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid,  $\gamma$ -amino butyric acid,  $\epsilon$ -amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid,  $\tau$ -butylglycine,  $\tau$ -butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form. Allelic variants of a protein sequence of a detectable protein used in the present invention are also encompassed within the scope of the invention.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby a marker protein having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as a marker protein having the substitution still is detectable Table 1 below provides a listing of examples of amino acids belonging to each class.

Table 1.	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

20

### Polynucleotides

cDNA sequences encoding the proteins of the present invention are provided. Polynucleotides of the present invention can be composed of either RNA or DNA. Preferably, the polynucleotides are composed of DNA. The subject invention also

encompasses those polynucleotides that are complementary in sequence to the polynucleotides disclosed herein.

Specifically exemplified are DNA sequences that encode for scubRFP from *Scolymia cubensis*; and g5.2 (cyan), mc6 (green) and R7 (green) proteins, from *Montastraea cavernosa*. These DNA sequences are set forth in SEQ. ID NOS. 3-6.

Sequences of the subject invention may utilize codons preferred for expression by the selected host strains. These sequences may also have sites for cleavage by restriction enzymes, and/or initial, terminal, or intermediate DNA sequences which facilitate construction of readily expressed vectors.

Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode the detectable proteins of the present invention. In addition, it is well within the skill of a person trained in the art to create alternative polynucleotide sequences encoding the same, or essentially the same, detectable proteins of the subject invention. These variant or alternative polynucleotide sequences are within the scope of the subject invention. As used herein, references to "essentially the same" sequence refers to sequences which encode amino acid substitutions, deletions, additions, or insertions which do not eliminate the detectability of the polypeptide encoded by the polynucleotides of the present invention. Allelic variants of the nucleotide sequences encoding a genetic marker protein of the invention are also encompassed within the scope of the invention.

The subject invention also concerns variants of the polynucleotides of the present invention that encode detectable proteins. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted. The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Polynucleotides and polypeptides of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used.

The subject invention also contemplates those polynucleotide molecules having sequences that are sufficiently homologous with the polynucleotide sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis *et al.* 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature ( $T_m$ ) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature,  $T_m$ , is described by the following formula (Beltz *et al.*, 1983):

$$T_m = 81.5 C + 16.6 \log[Na^+] + 0.41(\%G+C) - 0.61(\% \text{ formamide}) - 600/\text{length of duplex in base pairs.}$$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20$  C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

The polynucleotide sequences include the DNA strand sequence that is transcribed into RNA and the strand sequence that is complementary to the DNA strand that is transcribed. The polynucleotide sequences also include both full-length sequences as well as shorter sequences derived from the full-length sequences. The polynucleotide sequence includes both the sense and antisense strands either as individual strands or in the duplex.

#### Recombinant Hosts

Polynucleotide molecules containing DNA sequences encoding the colored and/or fluorescent proteins of the present invention can be introduced into a variety of host cells including bacterial cells, yeast cells, fungal cells, plant cells and animal cells. Methods by which the exogenous genetic material can be introduced into such host cells are well known in the art.

In one embodiment, the invention provides a bacteria cell capable of expressing the novel colored and fluorescent proteins.

Plants, plant tissues, and plant cells bred to contain, or transformed with, a polynucleotide of the invention are also contemplated by the present invention. In one embodiment, the polynucleotide encodes a detectable polypeptide shown in SEQ ID NOS. 7-10, or a functional fragment or variant thereof. Plants within the scope of the present invention include monocotyledonous plants, such as rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet; and dicotyledonous plants, such as peas, alfalfa, tomato, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple trees, grape, cotton, sunflower, and lettuce; and conifers. Techniques for transforming plant cells with a gene are known in the art and include, for example, *Agrobacterium* infection, biolistic methods, electroporation, calcium chloride treatment, *etc.* Transformed cells can be selected, redifferentiated, and grown into plants using standard methods known in the art. The progeny of any transformed plant cells or plants are also included within the scope of the present invention.

The subject invention also concerns non-human transgenic animals which have incorporated into the host cell genome a polynucleotide of the invention. Methods for producing transgenic animals, including mice, rats, pigs, sheep, cows, fish, and the like are well known in the art.



The subject invention also concerns methods for isolating transformants expressing a transgene. In one embodiment, an expression construct of the present invention comprising a transgene of interest operably linked to a nucleotide sequence encoding a detectable marker of the present invention is used to transform a cell. Methods for transforming cells are well known in the art. Transformed cells expressing the transgene are selected by identifying those cells expressing a genetic marker of the invention.

#### Expression Constructs

An expression construct of the invention typically comprises a structural gene sequence (encoding a protein), an antisense sequence, or other polynucleotide sequences, or a site for insertion of such sequences, operably linked to a polynucleotide of the present invention encoding a marker. The structural gene can be a gene encoding a protein from a prokaryotic or eukaryotic organism; for example, a human, mammal, insect, plant, bacteria, or virus. Proteins that can be encoded by a gene sequence include, but are not limited to, enzymes, hormones, cytokines, interleukins, receptors, growth factors, immunoglobulins, transcription factors, and *Bacillus thuringiensis* (*B.t.*) crystal toxin proteins. Sequences encoding *B.t.* proteins which have codon usage for preferential expression in plants are described in U.S. Patent Nos. 5,380,831; 5,567,862; 5,567,600; 6,013,523; and 6,015,891. An antisense sequence is a sequence wherein the RNA transcribed from the antisense sequence is at least partially complementary to RNA transcribed from a gene encoding a protein.

Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in, for example, bacterial host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a marker of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an

expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

For expression in prokaryotic systems, an expression construct of the invention can comprise promoters such as, for example, alkaline phosphatase promoter, tryptophan (trp) promoter, lambda P<sub>L</sub> promoter,  $\beta$ -lactamase promoter, lactose promoter, phoA promoter, T3 promoter, T7 promoter, or tac promoter (de Boer *et al.*, 1983).

Expression constructs for use in bacteria are given in SEQ ID NOS. 11-14, and the corresponding amino acid sequences are given in SEQ ID NOS. 15-18.

If the expression construct is to be provided in a plant cell, plant viral promoters, such as, for example, the cauliflower mosaic virus (CaMV) 35S (including the enhanced CaMV 35S promoter (see, for example U.S. Patent No. 5,106,739)) or 19S promoter can be used. Plant promoters, such as prolifera promoter, Ap3 promoter, heat shock promoters, T-DNA 1'- or 2'-promoter of *A. tumefaciens*, polygalacturonase promoter, chalcone synthase A (CHS-A) promoter from petunia, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter (Xu *et al.*, 1993), maize WipI promoter, maize trpA gene promoter (U.S. Patent No. 5,625,136), maize CDPK gene promoter, and RUBISCO SSU promoter (U.S. Patent No. 5,034,322) can also be used. Seed-specific promoters such as the promoter from a  $\beta$ -phaseolin gene (of kidney bean) or a glycinin gene (of soybean), and others, can also be used. Constitutive promoters (such as the CaMV, ubiquitin, actin, or NOS promoter), tissue-specific promoters (such as the E8 promoter from tomato), developmentally-regulated promoters, and inducible promoters (such as those promoters that can be induced by heat, light, hormones, or chemicals) are contemplated for use with the polynucleotides of the invention.

For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. If the cells are mammalian cells, then promoters such as, for example, actin promoter, metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early

promoter and SV40 late promoter, Lck promoter, BMP5 promoter, TRP-1 promoter, murine mammary tumor virus long terminal repeat promoter, STAT promoter, or an immunoglobulin promoter can be used in the expression construct. The baculovirus polyhedrin promoter can be used with an expression construct of the invention for expression in insect cells. Promoters suitable for use with an expression construct of the invention in yeast cells include, but are not limited to, 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase promoter, metallothionein promoter, alcohol dehydrogenase-2 promoter, and hexokinase promoter.

Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, signal peptide sequence, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient termination. Signal peptides are a group of short amino terminal sequences that encode information responsible for the relocation of an operably linked mature polypeptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting marker gene products to an intended cellular and/or extracellular destination through the use of operably linked signal peptide sequence is contemplated for use with the polypeptides of the invention. Enhancers are cis-acting elements that increase activity of a promoter and can also be included in the expression construct. Enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, maize shrunken-1 enhancer element, cytomegalovirus (CMV) early promoter enhancer element, and the SV40 enhancer element.

DNA sequences which direct polyadenylation of the mRNA encoded by the structural gene can also be included in the expression construct. The expression constructs of the invention can also include a polynucleotide sequence that directs transposition of other genes, *i.e.*, a transposon.

### Applications

There are many ways in which the novel proteins of the subject invention can be used. In one embodiment, the proteins can be used to identify cells. In these

methods the proteins can be used to express fluorescence in a cell. One use for this method is in pre-labeling isolated cells or a population of similar cells prior to exposing the cells to an environment in which different cell types are present. Detection of fluorescence in only the original cells allows the location of such cells to  
5 be determined and compared with the total population.

A second group of methods concerns the identification of cells that have been transformed with exogenous DNA of interest. Identifying cells transformed with exogenous DNA is required in many *in vitro* procedures as well as in *in vivo* applications such as gene therapy.

10 In one embodiment of the subject invention, a polynucleotide sequence encoding a protein of the subject invention is fused to a DNA sequence encoding a selected protein in order to directly label the encoded protein. Expressing such a fluorescent and/or colored protein in a cell results in the production of labeled  
15 proteins that can be readily detected. This is useful in confirming that a protein is being produced by a chosen host cell. It also allows the location of the selected protein to be determined.

Cells that have been transformed with exogenous DNA can also be identified without creating a fusion protein. Here, the method relies on the identification of cells that have received a plasmid or vector that comprises at least two transcriptional or  
20 translational units. A first unit encodes and directs expression of the desired protein, while the second unit encodes and directs expression of the detectable protein. Co-expression of the detectable protein from the second transcriptional or translational unit ensures that cells containing the vector are detected and differentiated from cells that do not contain the vector.

25 In methods to produce fluorescent molecular weight markers, a gene sequence is generally fused to one or more DNA sequences that encode proteins having defined amino acid sequences and the fusion proteins are expressed from an expression vector. Expression results in the production of fluorescent proteins of defined molecular weight or weights that may be used as markers (following calculation of  
30 the size of the complete amino acid sequence).

Amino acid replacements that produce different color forms permit simultaneous use of multiple reporter genes. Different colored proteins can be used to identify multiple cell populations in a mixed cell culture or to track multiple cell

types, enabling differences in cell movement or migration to be visualized in real time without the need to add additional agents or fix or kill the cells.

Other options include tracking and determining the ultimate location of multiple proteins within a single cell, tissue or organism; differential promoter analysis in which gene expression from two different promoters is determined in the  
5 same cell, tissue or organism; and FACS sorting of mixed cell populations.

The techniques that can be used with spectrally separable proteins are exemplified by confocal microscopy, flow cytometry, and fluorescence activated cell sorting (FACS) using modular flow, dual excitation techniques.

10 In one embodiment, the subject invention concerns polynucleotides comprising an in-frame fusion of nucleotide sequences encoding multiple genetic markers. For example, a polynucleotide of the invention may comprise a first nucleotide sequence that is operably linked in-frame to a second nucleotide sequence. The polynucleotide encodes the amino acid sequences of the detectable protein and  
15 another genetic marker such that the genetic markers are in direct contact with one another, *i.e.*, where the last amino acid of the fluorescent genetic marker is immediately contiguous with the first amino acid of the other genetic marker, or they can be separated by a peptide linker sequence, for example, as described in U.S. Patent Nos. 5,891,680 and Li *et al.*, 2001, that do not substantially alter functional  
20 activity of the genetic markers.

The subject invention also concerns kits comprising in one or more containers and a polynucleotide and/or protein of the present invention.

Additional useful applications of the technology described herein include, but are not limited to, the following:

25

FRET – Fluorescence Resonant Energy Transfer: This technique allows observation and quantification of molecular interactions. It requires at least two fluorescent proteins of different colors. Currently the most widely used pair is CFP and YFP (mutated variants of GFP); the proteins of the subject invention may be substituted for  
30 either or both of them.

References:

1. Hanson, M. R. and R. H. Kohler. 2001. GFP imaging: methodology and application to investigate cellular compartmentation in plants. *J Exp Bot* 52: 529-539.
2. Pollok, B. A. and R. Heim. 1999. Using GFP in FRET-based applications. *Trends Cell Biol* 9: 57-60.
3. Schuttrigkeit, T. A., U. Zachariae, T. von Feilitzsch, J. Wiehler, J. von Hummel, B. Steipe and M. E. Michel-Beyerle. 2001. Picosecond time-resolved FRET in the fluorescent protein from *Discosoma Red* (wt-DsRed). *Chemphyschem* 2: 325-328.
4. Hillisch, A., M. Lorenz and S. Diekmann. 2001. Recent advances in FRET: distance determination in protein-DNA complexes. *Curr Opin Struct Biol* 11: 201-207.

FRAP – Fluorescence Redistribution After Photobleaching: This technique quantifies the dynamics of tagged molecules or the reporter molecules themselves. It involves in photobleaching (burning out) of all the fluorescent molecules within a small area by intense excitation light and monitoring the process of fluorescence recovery within this area (due to migration of tagged molecules from adjacent areas).

#### References:

1. Reits, E. A. and J. J. Neeffjes. 2001. From fixed to FRAP: measuring protein mobility and activity in living cells. *Nat Cell Biol* 3: E145-147.
2. Houtsmuller, A. B. and W. Vermeulen. 2001. Macromolecular dynamics in living cell nuclei revealed by fluorescence redistribution after photobleaching. *Histochem Cell Biol* 115: 13-21.

“Fluorescent timer” applications: one of the proteins exemplified herein – scubRFP – due to its natural spectroscopic properties, can be used as a reporter that changes color with time. Such reporters make it possible to estimate the time elapsed since the reporter protein was synthesized by quantifying its color. In addition, since the maturation speed (the rate of conversion from green to red) in scubRFP can be increased by UV-A light, it is possible to adjust its timing scale: experiments that need timing in shorter intervals may use appropriate background UV illumination to speed up the green-to-red conversion.

## References:

1. Terskikh, A. V., A. Fradkov, A. Zarskiy, A. V. Kaja, M. Matz, S. Kim, I. Weissman and P. Siebert. 2000. "Fluorescent timer": Protein that changes color over time. *Molecular Biology of the Cell* 11: 648.
- 5 2. Verkhusha, V. V., H. Otsuna, T. Awasaki, H. Oda, S. Tsukita and K. Ito. 2001. An enhanced mutant of red fluorescent protein DsRed for double labeling and developmental timer of neural fiber bundle formation. *Journal of Biological Chemistry* 276: 29621-29624.

10 "Light-inducible fluorescence": since the red fluorescence of scubRFP can be induced by exposure to UV-A light, it is possible to use this protein as a light-inducible reporter. Such a reporter can be used for studying molecular dynamics, in a way that is analogous to FRAP (see above). A small area can be irradiated by the fluorescence-inducing light, after which the process of redistribution of active fluorescent  
15 molecules from the irradiated spot can be followed.

## References:

1. Ando, R., H. Hama, M. Yamamoto-Hino, H. Mizuno and A. Miyawaki. 2002. An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. *Proceedings of the National Academy of Sciences of the United States of America* 99: 12651-12656.
- 20 2. Patterson, G. H. and J. Lippincott-Schwartz. 2002. A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* 297: 1873-1877.
3. Chudakov, D. M., V. V. Belousov, A. G. Zarskiy, V. V. Novoselov, D. B. Staroverov, D. B. Zorov, S. Lukyanov and K. A. Lukyanov. 2003. Kindling  
25 fluorescent proteins for precise in vivo photolabeling (vol 21, pg 191, 2003). *Nature Biotechnology* 21: 452-452.

Coloring of biological objects for decorative and other non-scientific purposes. Examples: producing decorative fish for aquariums; coloring of fur, wool and milk by  
30 means of genetic modifications of appropriate animals; and coloring of decorative plants. Such uses can be implemented by a person skilled in the art having the benefit of the teachings of the current disclosure.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

5

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### 10 Example 1 — Bacterial Expression Construct

As illustrated in Figure 1, to prepare a bacterial expression construct, the ORF of the target detectable protein was amplified by means of polymerase chain reaction (PCR), using primers corresponding to the beginning and end of the protein's ORF. The upstream primer carried a 5'-heel ttgattgattgaaggagaaatatcATG (SEQ ID NO:1),  
15 which encoded three termination codons in three frames (bold), followed by the ribosome binding site (underlined), 6 spacer bases and initiation ATG codon.

The downstream primer encoded a 6xHis tag in place of the original termination codon (the heel sequence was 5'-tta tta gtg atg gtg atg gtg atg (SEQ ID NO:2)), to facilitate protein purification by means of metal-affinity chromatography.

20 The products of amplification were cloned into pGEM-T vector (Promega) using manufacturer-provided reagents and protocol. The expressing clones were identified after overnight growth of the colonies by their fluorescent appearance.

#### Example 2 — Additional Proteins and Polynucleotides

25 The subject invention also provides proteins from *Acropora* ("staghorn corals") and *Agarica fragilis* ("fragile saucer coral"), as well as polynucleotides encoding these proteins.

In one embodiment, the invention provides nucleotide sequences of the inserts in pGEM-T vector (Promega), the conceptual translations of these inserts, and special  
30 properties of purified protein products.

The vector constructs are shown in SEQ ID NOs.:19-44. The encoded proteins are shown in SEQ ID NOs.:45-70. The open reading frames encoding the



proteins of SEQ ID NOs.: 45-70 are shown in SEQ ID NOs.:71-96. The spectral characteristics of the proteins are shown in Figures 4-29.

### Example 3 — Excitation and emission spectra of the detectable proteins

5       The excitation spectra were measured from the proteins purified after bacterial expression. The spectra are shown in Figures 2-29. Emission spectra (dotted lines) were measured using USB2000 uv-vis spectrometer (Ocean Optics), excitation spectra (solid lines) – using spectrofluorometer LS-50B (Perkin Elmer). The indicated positions of excitation and emission maxima are accurate within 5 nm.

### Example 4 — Multiple Marker Constructs

10       There are several advantages associated with the use of fusion markers, including: 1) achievement of combined functionalities in a single transcription unit, 2) reduced usage of genetic elements, such as promoters and terminators, for expressing  
15       multiple marker genes, 3) reduced overall length of insertion sequences that may lead to increased transformation efficiency, and most importantly 4) elimination of molecular interactions between adjacent genetic elements. Such unwanted interactions are frequently encountered when multiple expression units associated with different marker genes are used simultaneously and often complicate the  
20       interpretation of expression results.

      In an effort to improve marker functionality and versatility, several translational fusions between two genetic markers have been developed. Datla *et al.* (1991; U.S. Patent No. 5,639,663) created a bifunctional fusion between GUS and neomycin phosphotransferase (NPTII) to provide a biochemically assayable reporter  
25       activity and a conditionally selectable growth advantage for use in plant transformation. Another bifunctional fusion, between GUS and GFP, was also developed to provide both indicative and assayable reporter activities for monitoring transient and stable transgene expression in plant cells (Quaedvlieg *et al.*, 1998). More recently, Li *et al.* (2001) constructed a bifunctional fusion between GFP and  
30       NPTII and successfully used this marker for continuous analysis of promoter activity and transgene expression in transgenic grape plants throughout the entire process of plant development.

Small portions of a protein that provide unique functions such as protein/DNA/substrate binding activity can be inserted into another heterologous protein to create a hybrid fusion with enhanced functionality and utility. In other cases, an entire gene or protein of interest has been fused in-frame to another heterologous gene or protein to form a double fusion to provide combined functionalities. Production of multiple proteins using fusion constructs composed of two genes from transgenic plants has been demonstrated previously (U.S. Patent No. 6,455,759).

In one embodiment, the subject invention provides cells transformed with a polynucleotide of the present invention comprising an in-frame fusion of nucleotide sequences encoding multiple markers. Preferably, the polynucleotide sequence is provided in an expression construct of the invention. The transformed cell can be a prokaryotic cell, for example, a bacterial cell such as *E. coli* or *B. subtilis*, or the transformed cell can be a eukaryotic cell, for example, a plant or animal cell. Animal cells include human cells, mammalian cells, avian cells, fish cells and insect cells. Mammalian cells include, but are not limited to, COS, 3T3, and CHO cells.

Genetic markers that can be used in conjunction with the detectable proteins of the present invention are known in the art and include, for example, polynucleotides encoding proteins that confer a conditionally selective growth advantage, such as antibiotic resistance and herbicide-resistance; polynucleotides encoding proteins that confer a biochemically assayable reporter activity; and polynucleotides encoding proteins that confer an indicative reporter activity. Examples of polynucleotides encoding proteins providing antibiotic resistance include those that can provide for resistance to one or more of the following antibiotics: hygromycin, kanamycin, bleomycin, G418, streptomycin, paromomycin, and spectinomycin. Kanamycin resistance can be provided by neomycin phosphotransferase (NPTII). Examples of polynucleotides encoding proteins providing herbicide resistance include those that can provide for resistance to phosphinothricin acetyltransferase or glyphosate. Examples of genetic markers that confer assayable or indicative reporters activity that can be used in the present invention include, but are not limited to, polynucleotides encoding  $\beta$ -glucuronidase (GUS),  $\beta$ -galactosidase, chloramphenicol acetyltransferase (CAT), luciferase, nopaline synthase (NOS), and green fluorescence protein (GFP).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

We claim:

1. A protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70 or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within  $\pm 10$  nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.
2. The protein, according to claim 1, wherein said protein has an amino acid sequence selected from the group consisting of SEQ. ID NOs: 7-10 and SEQ ID NOs: 45-70.
3. The protein, according to claim 1, wherein said protein has an amino acid sequence selected from the group consisting of SEQ. ID NOs: 7-10.
4. The protein, according to claim 1, wherein said protein has SEQ ID NO:10.
5. A polynucleotide sequence that encodes a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70, or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within  $\pm 10$  nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.
6. The polynucleotide sequence, according to claim 5, wherein said polynucleotide has a sequence is selected from the group consisting of SEQ. ID NOs: 3-6 and SEQ ID NOs: 71-96.

7. A polynucleotide sequence selected from the group consisting of SEQ ID NOs: 11-14 and SEQ ID NOs: 19-44.

8. A protein selected from the group consisting of the proteins expressed when a bacterium is transformed with a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 11-14 and SEQ ID NOs: 19-44.

9. Use of a protein, wherein said protein comprises a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70, or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within  $\pm 10$  nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70, for monitoring gene expression; as a tag for tracing the location of proteins; as a reporter molecule in a protein-based biosensor; or as a pigment for modifying the color, or fluorescence, or both, of living tissue.

10. The use, according to claim 9, wherein a polynucleotide that encodes said protein is fused with a polynucleotide that encodes a protein of interest.

11. A cell transformed to express a heterologous polynucleotide wherein said heterologous polynucleotide encodes a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70 or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within  $\pm 10$  nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.

12. The cell, according to claim 11, wherein said cell is a plant cell.

13. The cell, according to claim 11, wherein said cell is a fish cell.

14. A polynucleotide encoding multiple markers wherein at least one of said markers is selected from the group consisting of a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70 or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within  $\pm 10$  nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.

15. The polynucleotide, according to claim 14, wherein one of said markers is GUS.

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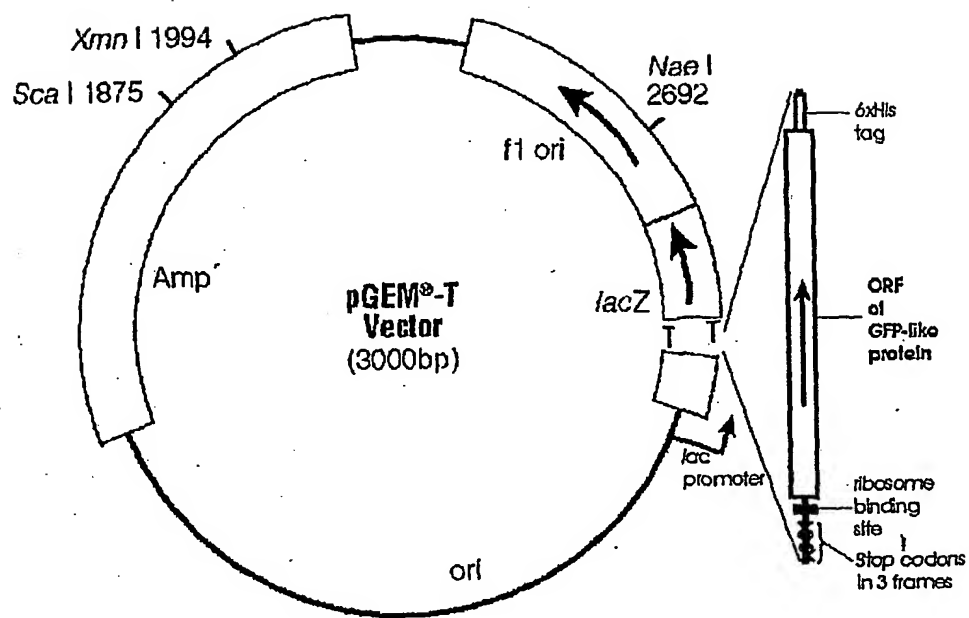


FIG. 1

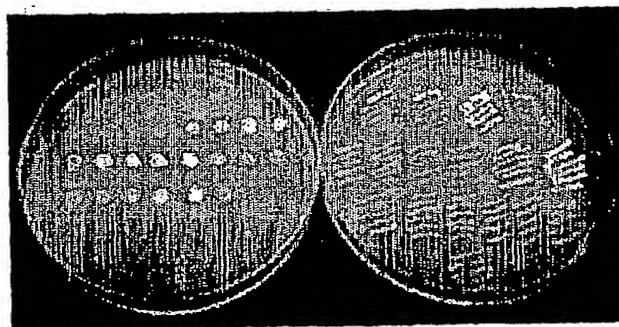


FIG. 2

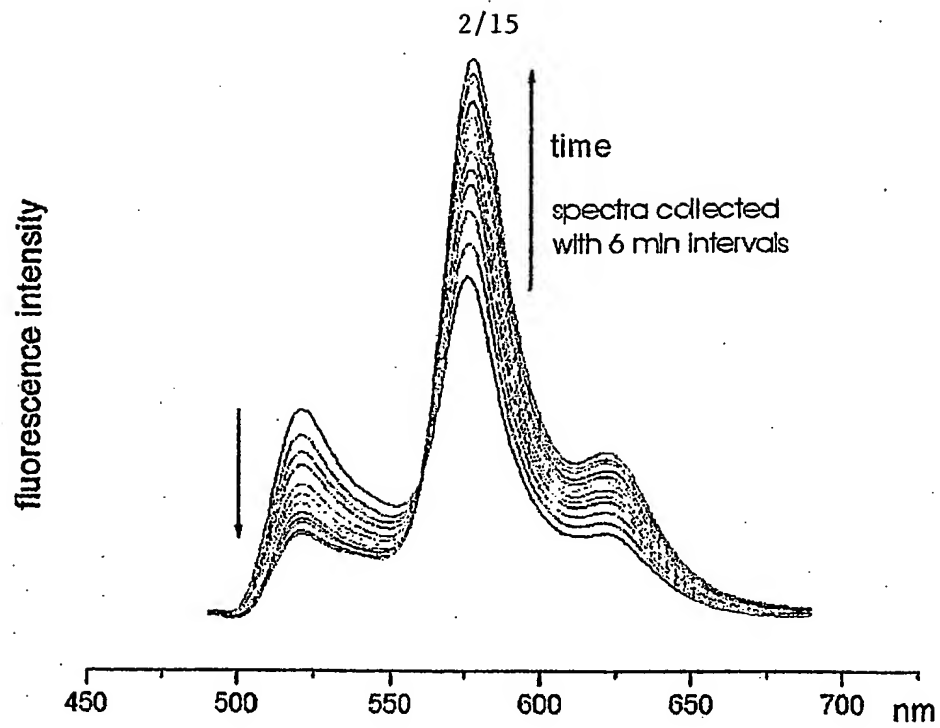


FIG. 3A

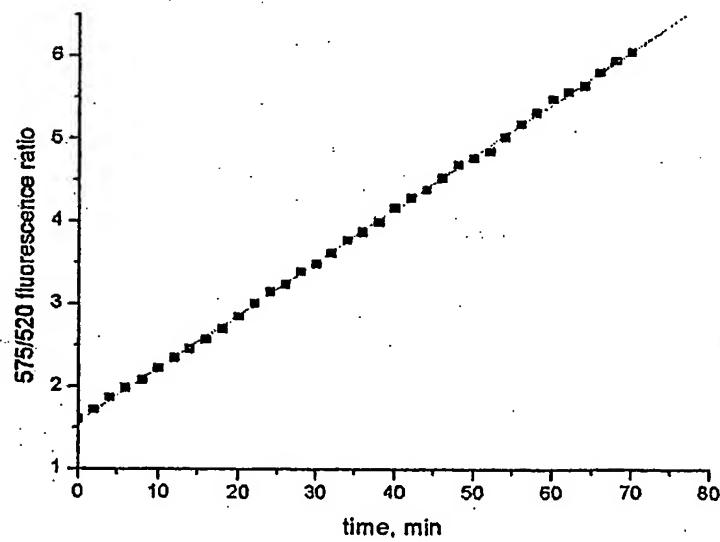


FIG. 3B



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A. aculeus 1-1 (green)

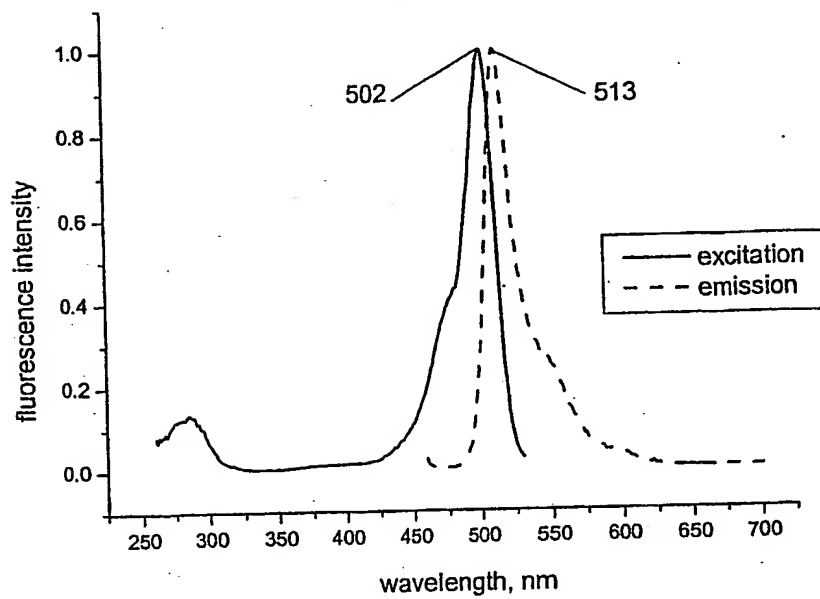


FIG. 4

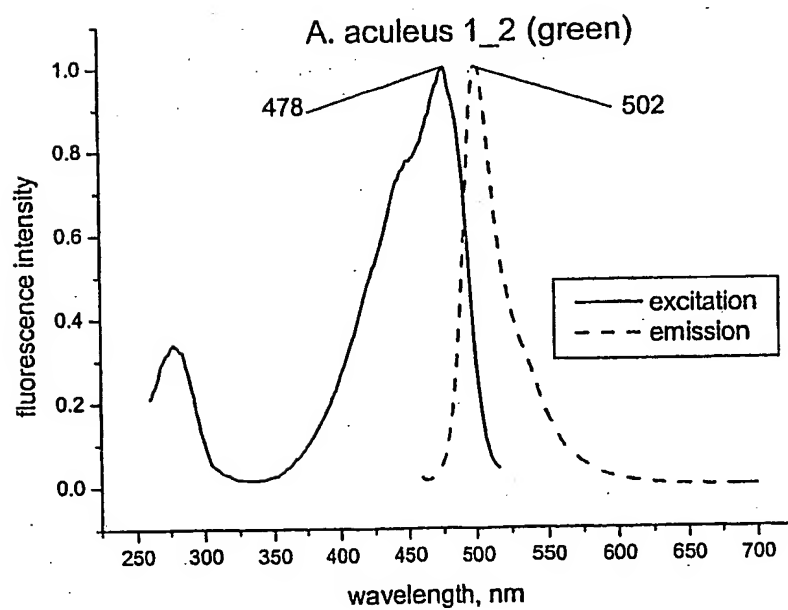


FIG. 5

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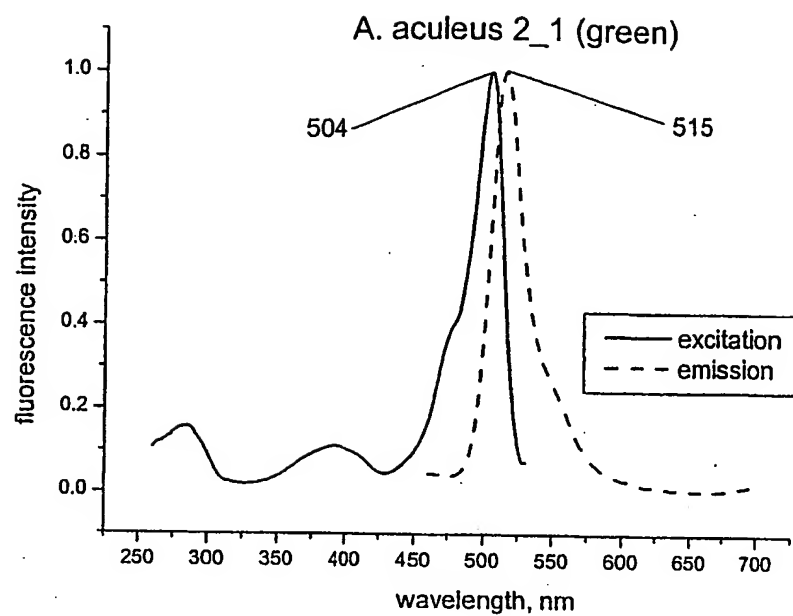


FIG. 6

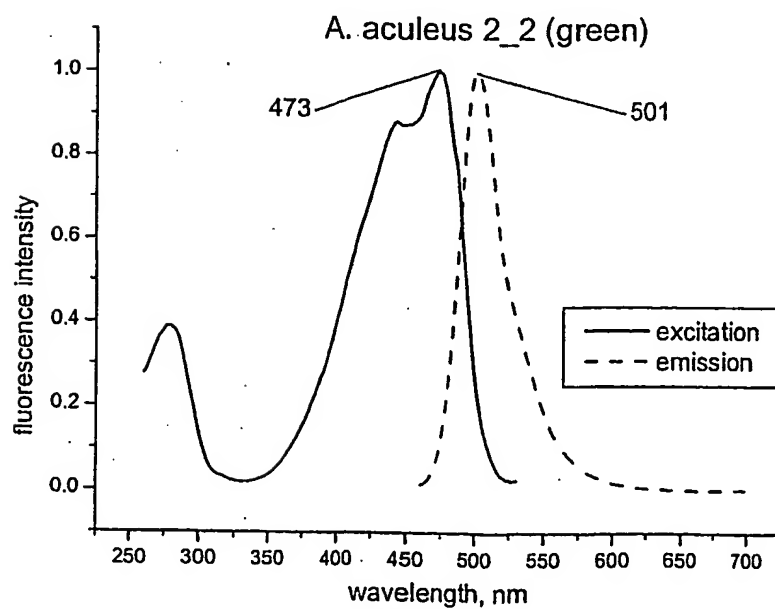


FIG. 7

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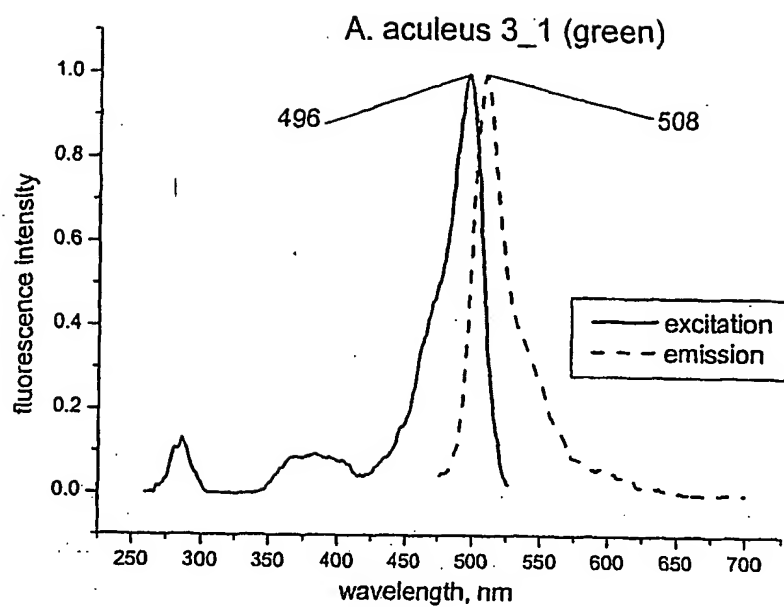


FIG. 8

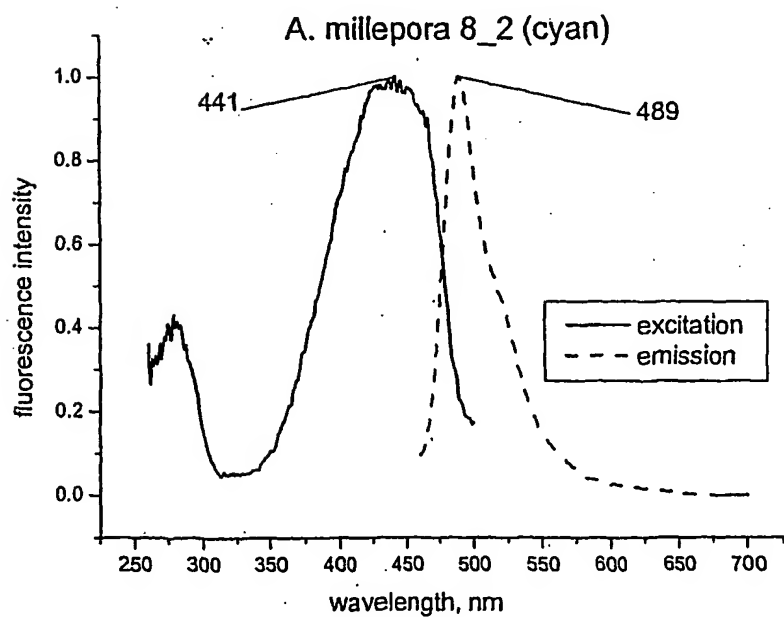


FIG. 9

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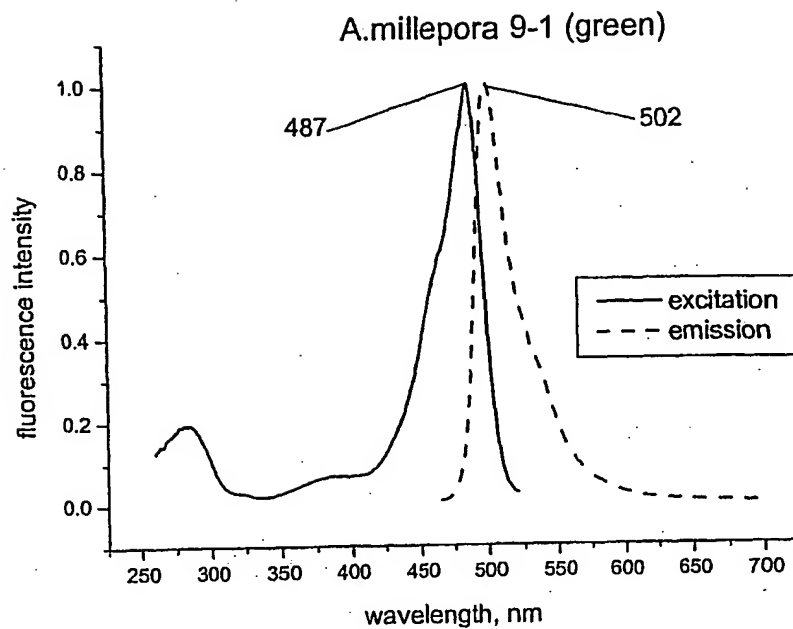


FIG. 10

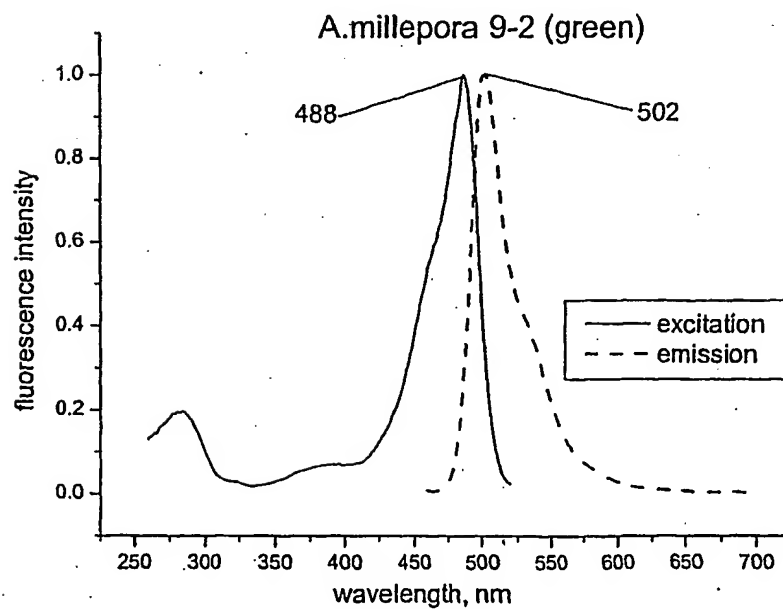


FIG. 11

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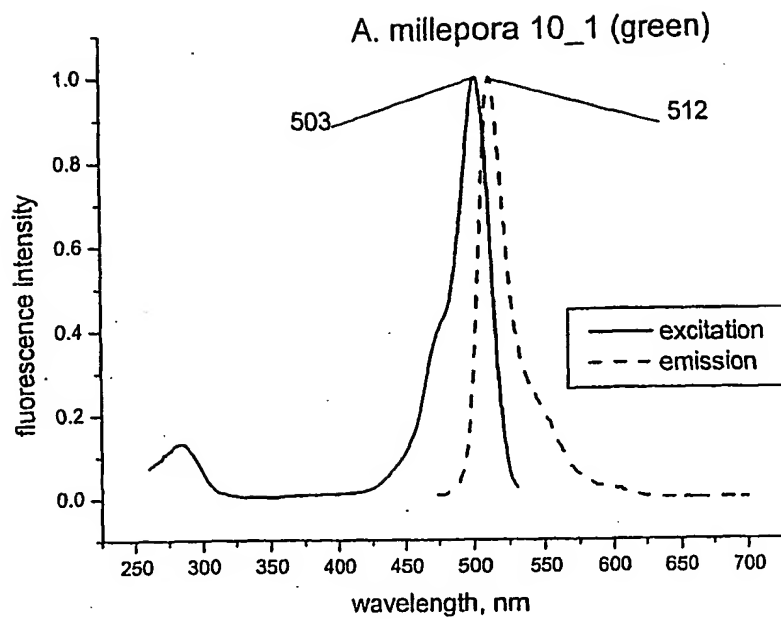


FIG. 12

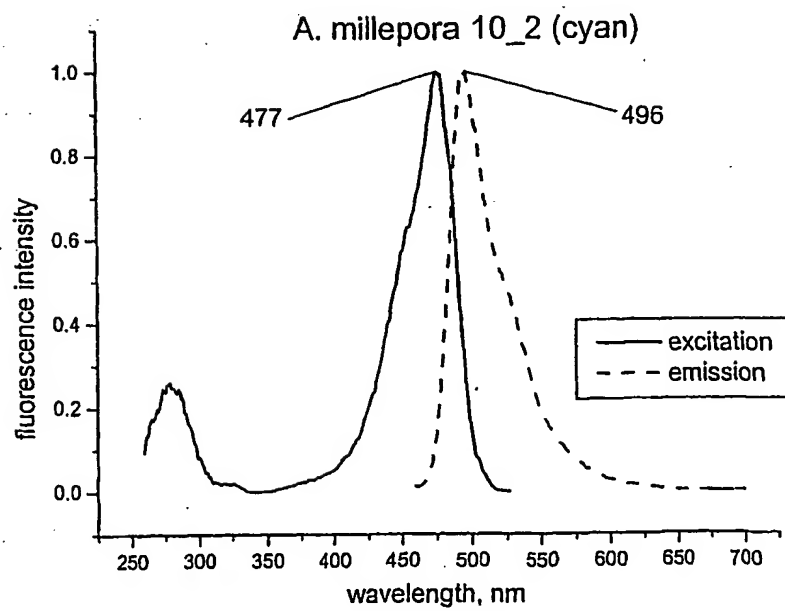


FIG. 13

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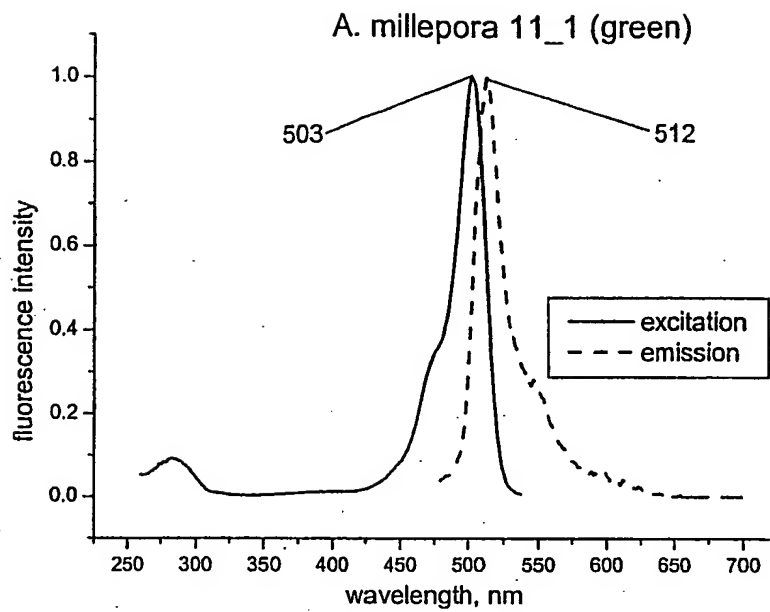


FIG. 14

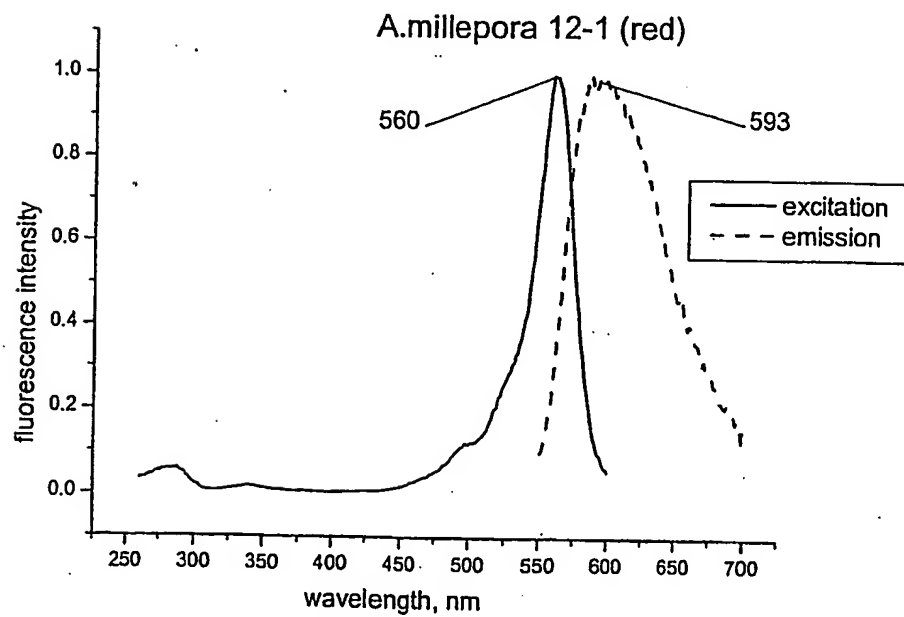


FIG. 15

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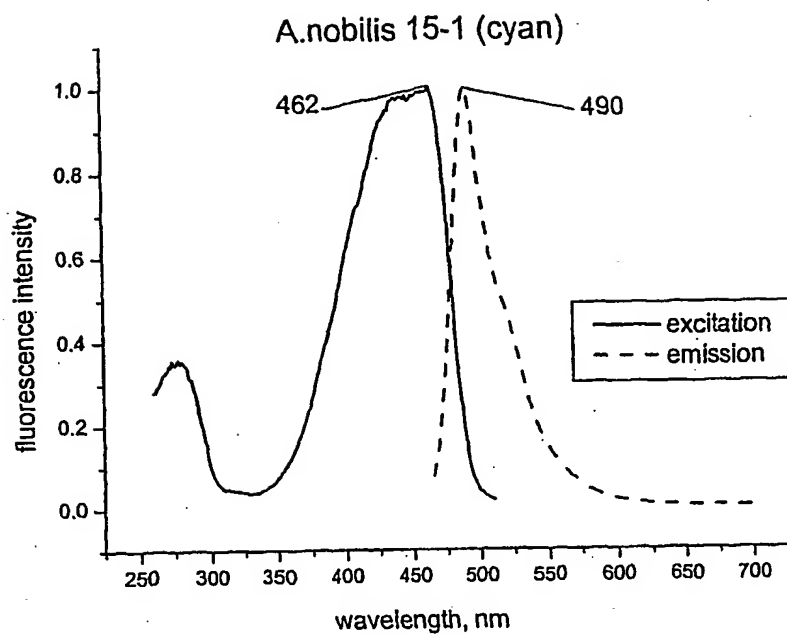


FIG. 16

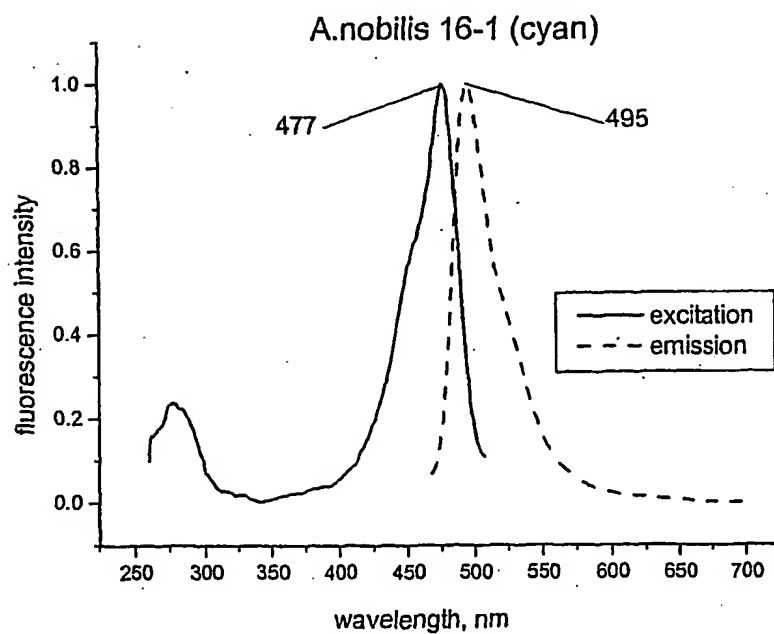


FIG. 17

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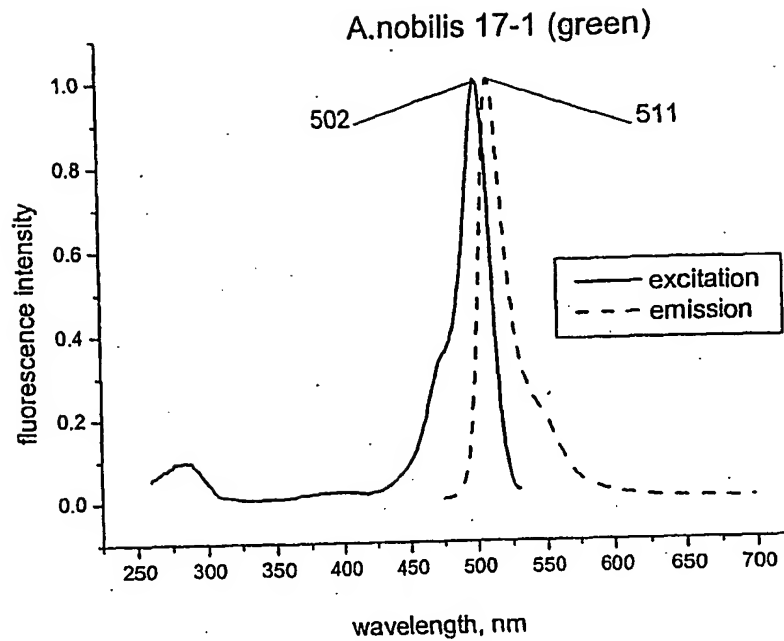


FIG. 18

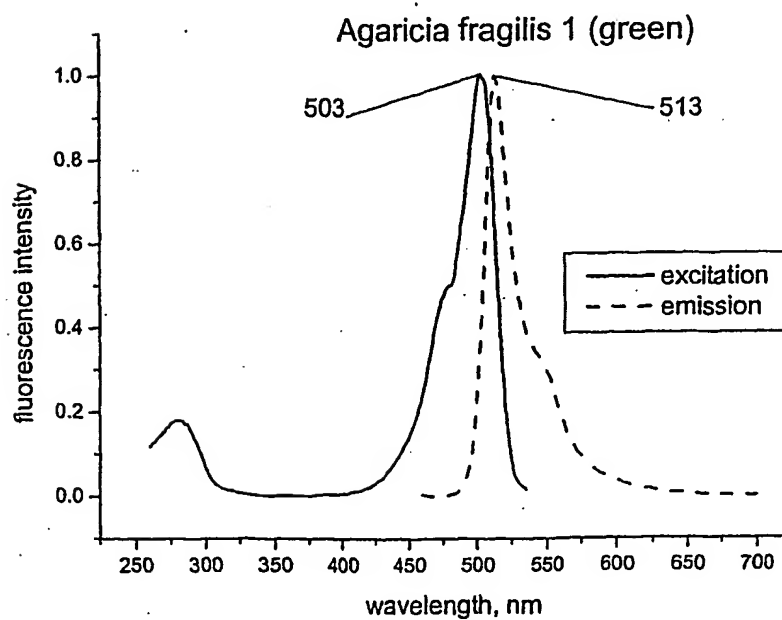


FIG. 19



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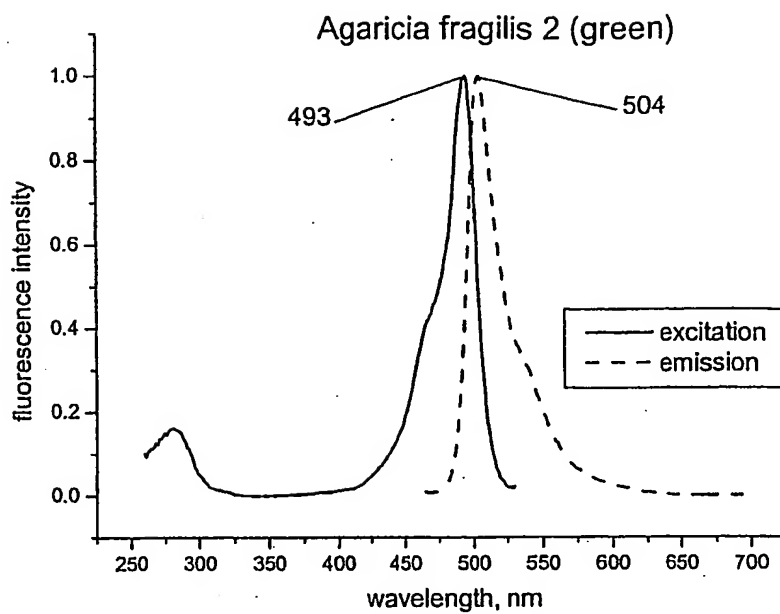


FIG. 20

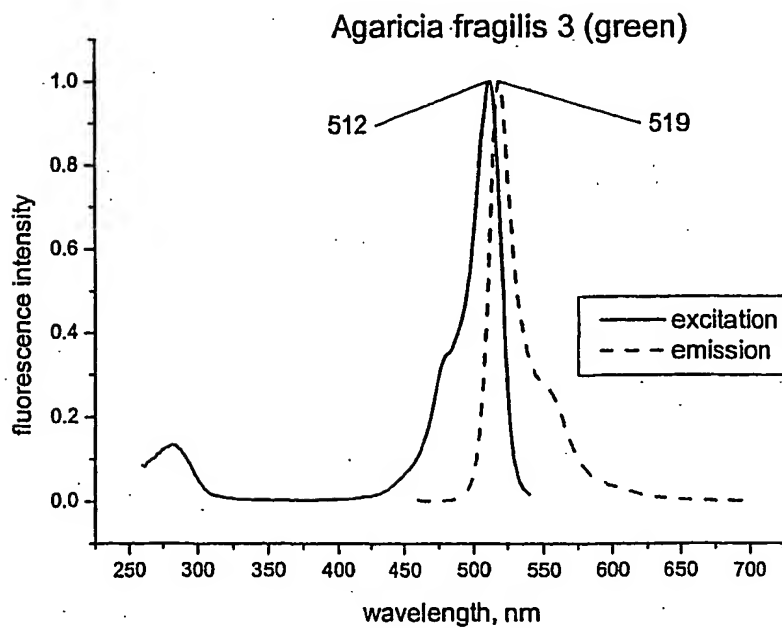


FIG. 21

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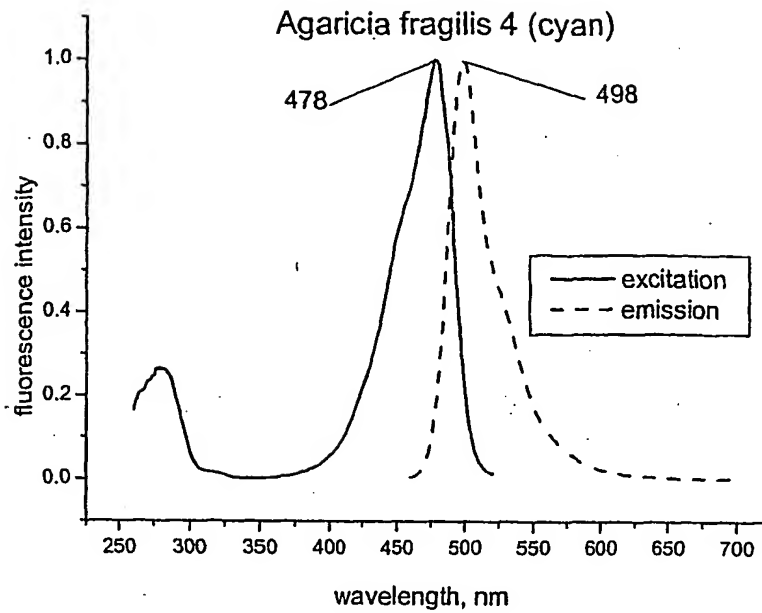


FIG. 22

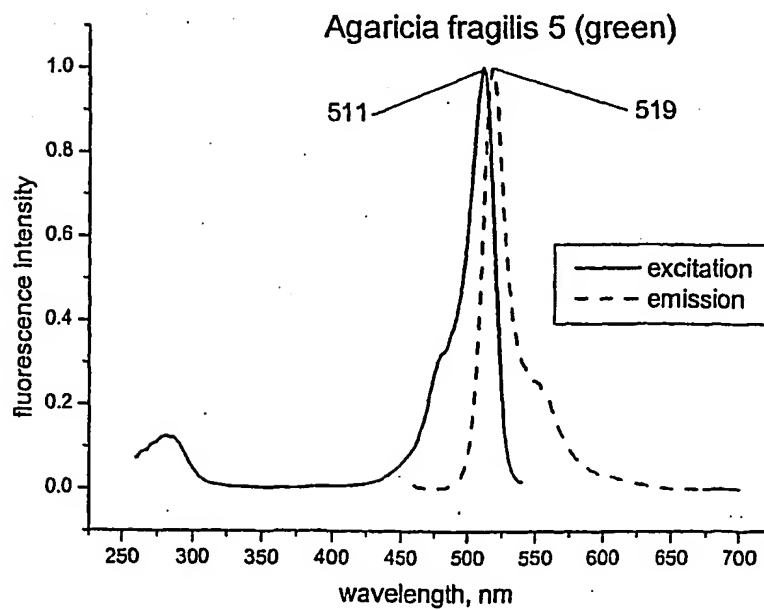


FIG. 23

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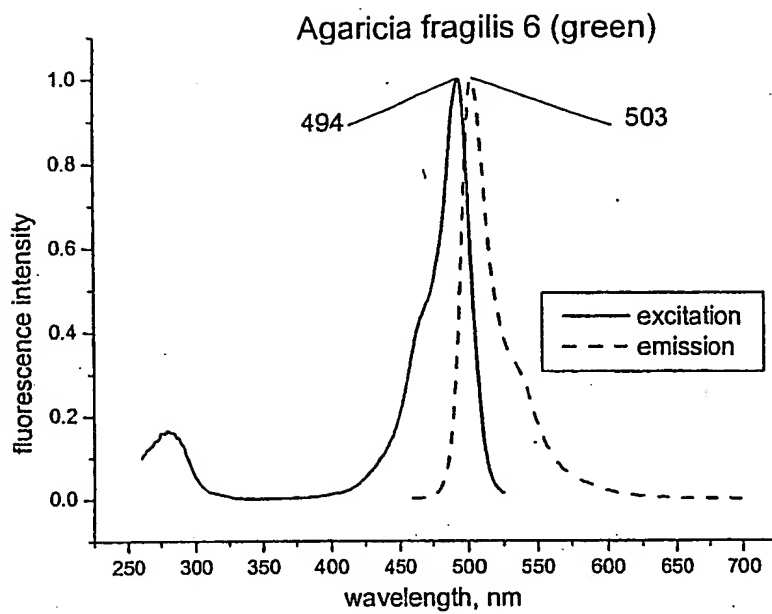


FIG. 24

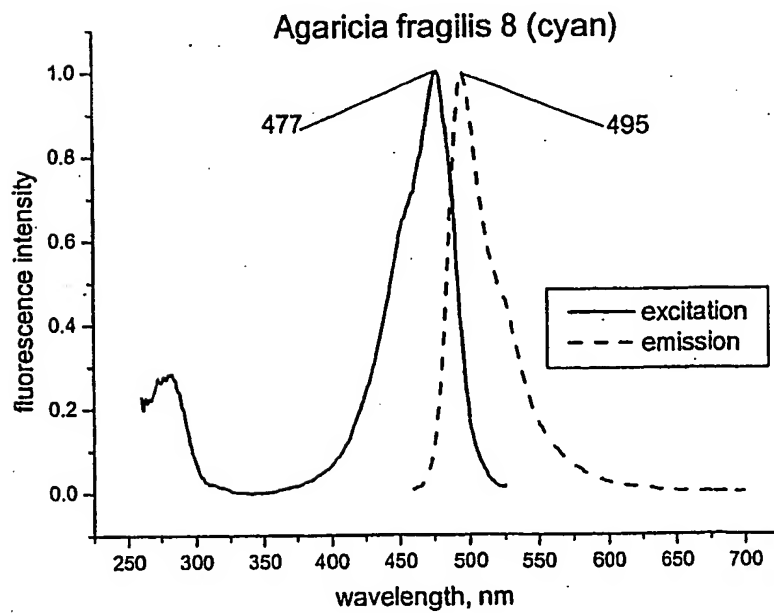


FIG. 25

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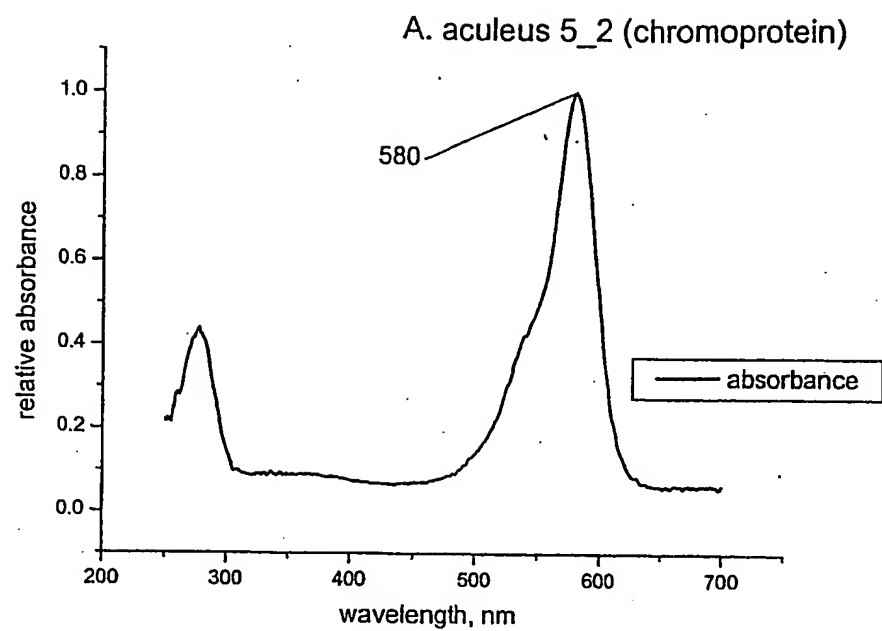


FIG. 26

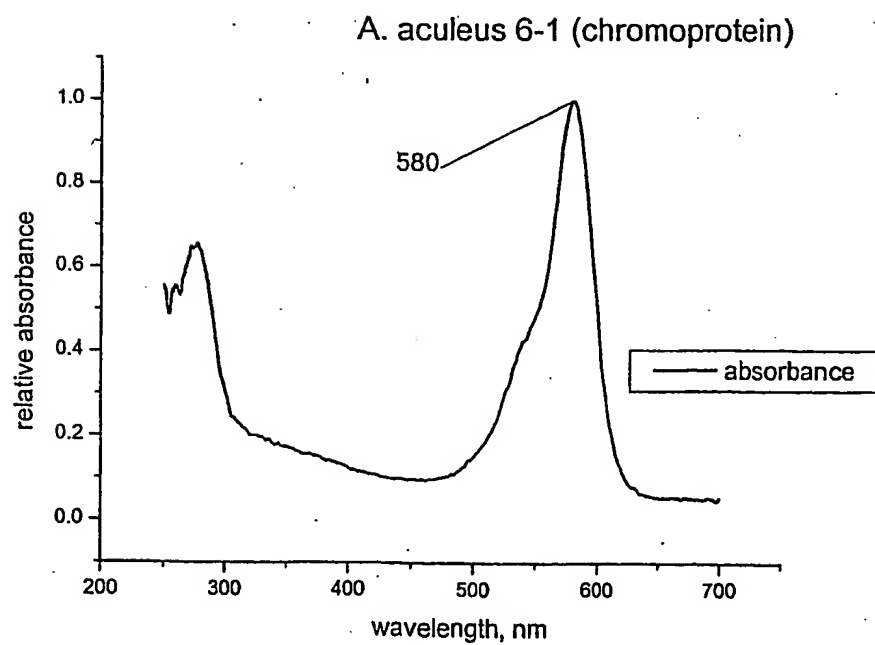


FIG. 27

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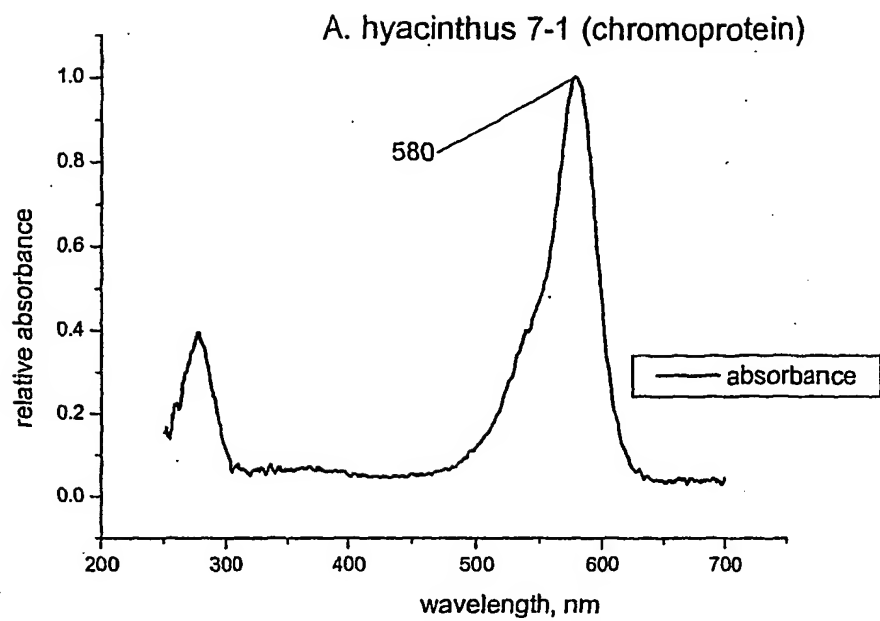


FIG. 28

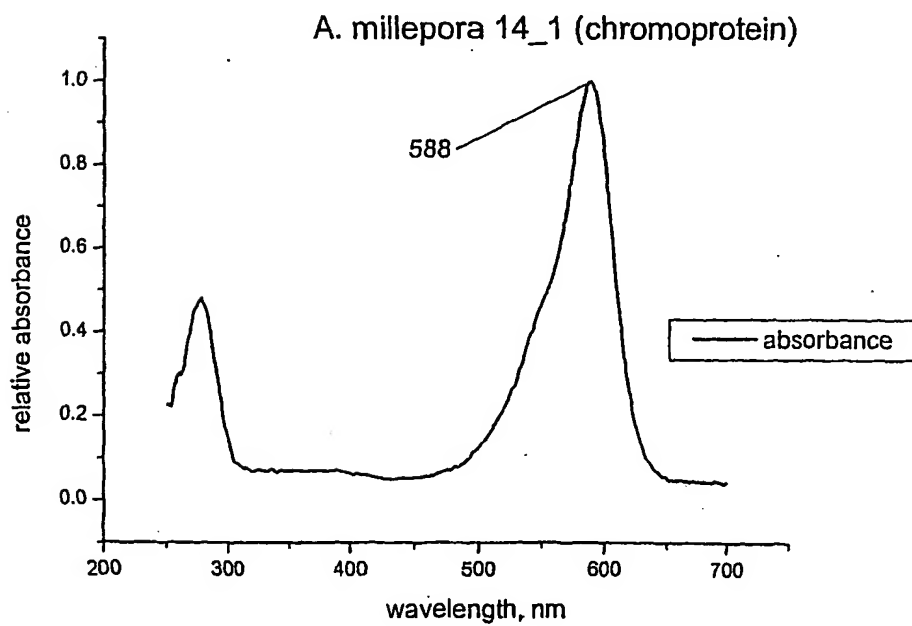


FIG. 29

## SEQUENCE LISTING

<110> University of Florida Research Foundation, Inc.  
 Matz, Mikhail  
 Kelmanson, Ilya  
 Salih, Anya  
 Meleshkevitch, Ella

<120> Novel Fluorescent and Colored Proteins, and Polynucleotides that  
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<130> UF-364XC1

<150> US 60/472,196

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20 25 30

Pro Phe Gln Gly Thr His Thr Ile Thr Leu Lys Val Lys Glu Gly Gly  
35 40 45

Pro Leu Pro Phe Pro Tyr Asp Ile Leu Thr Thr Ala Phe Gln Tyr Gly  
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Arg Asp Ile Pro Asp Tyr Phe Lys  
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Phe Glu  
85 90 95

Asp Gln Gly Ile Cys Thr Val Thr Ser Asp Ile Lys Leu Glu Gly Asp  
100 105 110

Cys Phe Phe Tyr Glu Ile Arg Phe Tyr Gly Val Asn Phe Pro Ser Asn  
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu  
130 135 140

Asn Met Tyr Val Arg Asp Gly Val Leu Leu Gly Asp Val Asn Arg Thr  
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Leu Leu Leu Glu Gly Asp Lys His His Arg Cys Asn Phe Arg Ser Thr  
165 170 175

Tyr Arg Ala Lys Lys Gly Val Val Leu Pro Glu Tyr His Phe Val Asp  
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 100 105 110  
 Cys Phe Ile Tyr Glu Ile Arg Tyr Asp Gly Val Asn Phe Pro Pro Ser  
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 Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu  
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 Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala  
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 Lys  
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 35 40 45  
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 65 70 75 80  
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Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr  
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Tyr Lys Ala Lys Lys Asp Val His Leu Pro Asp Tyr His Tyr Val Asp  
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Met Val Gly Asp Thr Phe His Asn Glu Val Gln Phe Tyr Gly Val Asn  
115 120 125

Phe Pro Pro Asn Gly Pro Val Met Gln Arg His Thr Val Lys Trp Glu  
130 135 140

Pro Ser Thr Glu Lys Ile Tyr Val Arg Asp Gly Val Leu Thr Gly Asp  
145 150 155 160

Ile Thr Met Ala Leu Leu Leu Lys Gly Gly Thr His Tyr Arg Cys Asp  
165 170 175

Phe Arg Thr Thr Tyr Lys Ala Lys Glu Lys Gly Pro Lys Phe Pro Gly  
180 185 190

Tyr His Leu Val Asp His Cys Ile Glu Ile Thr Ser His Asp Lys Asp  
195 200 205

Tyr Asn Val Val Glu Leu Tyr Glu His Ala Val Ala His Ser Gly Leu  
210 215 220

Pro Asp Ser Ala Asn Arg  
225 230

<210> 11

<211> 726

<212> DNA

<213> Montastraea cavernosa

<400> 11

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cctttccagg gaacacacac tataaccctt aaagtcaaag aaggggggacc tctgcctttc	180
ccttacgaca tcttgacaac agcattccag tacggcaaca ggggtattcac caaataccca	240
agagacatac cagactatth caagcagtcg tttcctgagg ggtattcctg ggaaagaagc	300
atgactttcg aagaccaggg catttgcacc gtcacaagcg acataaagtt ggaaggcgac	360
tgttttttct acgaaattcg attttatggg gtgaactttc cctccaatgg tccagttatg	420
cagaagaaga cgctgaaatg ggagccatcc actgagaata tgtacgtgcg tgatggagtg	480
ctactggggg atgttaacag gactctgttg cttgaaggag ataaacatca ccgatgtaac	540
ttcagaagta cttacagggc gaagaagggt gtcgtgttgc cagaatatca ctttgtggac	600
caccgaattg aaattctgag ccatgacaaa gattacaaca ccgttgaggt gtatgagaat	660
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cactaa	726

<210> 12

<211> 729

<212> DNA

&lt;213&gt; Montastraea cavernosa

&lt;400&gt; 12

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cctttcgagg gaacacagac tataaacctt acagtcaaag aaggcggacc tctgcctttt      180
gcttacgata tcttgacagc agcattccag tacggcaaca gggcattcac caaatacca      240
agagacatag cagactatth caagcagtct tttcctgagg ggtattcctg ggaacgaagc      300
atgacttatg aagaccaggg catttgcac atcaagagcg acataagaat ggaaggcgac      360
tgctttatct atgaaattcg atatgatggg gtgaactttc cccaagtgg tccagttatg      420
caaaagaaga cgctgaaatg ggagccatcc actgagaaaa tgtatgtgcg tgatggagtg      480
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caccgcattg agattttgag ccatgacaat gactacaaca ccgtaaagct gtctgagaat      660
gccgaggctc gctattctat gctgccgagt caggccaagg aaagtgcaca tcaccatcac      720
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&lt;210&gt; 13

&lt;211&gt; 729

&lt;212&gt; DNA

&lt;213&gt; Montastraea cavernosa

&lt;400&gt; 13

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cctttcgagg gaacgcagac tataaacctt acagtcaaag aaggcgcacc tctccctttt      180
gcttacgaca tcttgacatc agcattccag tatggcaaca ggtattcac caaatatcca      240
gacgatatac cagactatth caagcagacg tttcctgaag ggtattcgtg ggagcgaatc      300
atggccttat aagaccagag tatttgcacg gccacaagcg acataaaaat ggaaggcgac      360
tgttttatct acgaaattca atttcatggg gtgaactttc cacccaatgg tccagttatg      420
cagaagaaga cgctgaaatg ggaaccatcc accgagaaaa tgtatgtgcg tgatggagtg      480
ctgaagggtg atgttaacat ggctctgttg cttgaaggag gtggccatta ccgatgtgac      540
ttcagaagta cttacaaagc gaagaaggat gttcatttgc cagactatca ctacgtggac      600
caccgcattg agattttgag ccatgacaaa gattacaaa atgttacgct gtatgagcat      660
gccaaagctc gctattctat gctgccgagt aaggccaagg aaagtgcaca tcaccatcac      720

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catcactaa

729

&lt;210&gt; 14

&lt;211&gt; 741

&lt;212&gt; DNA

&lt;213&gt; Scolymia cubensis

&lt;400&gt; 14

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ggtgatggca ctcttttga gggaaaacag actttacacc tcaaggtcaa agagggcgca      180
cctctgcctt ttgcctacga tctctgaca acagctcttc attacggaaa cagggtattc      240
gtcgaatacc cagaaaacat cccagactat ttcaagcagt cgttccctaa gggatattca      300
tgggaaagaa gcctaacttt cgaagacggg ggaatttgca tcgccagaag cgacatcaaa      360
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ggctctgtta tgcagaggca cacggtgaaa tgggagccat cactgagaa gatttatgtg      480
cgtgatggag tgttgacggg tgatattacc atggctctgt tgcttaaagg aggtacccat      540
taccgatgtg acttcagaac tacttataaa gctaaggaga agggtcctaa gttcccaggc      600
tatcaccttg tcgatcattg tattgagatt acaagccatg acaaagatta caacgtgggt      660
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&lt;210&gt; 15

&lt;211&gt; 234

&lt;212&gt; PRT

&lt;213&gt; Montastraea cavernosa

&lt;400&gt; 15

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Met Ser Val Ile Lys Ser Asp Met Lys Ile Lys Leu Pro Met Glu Gly
1           5           10          15
Thr Val Asn Gly His Lys Phe Val Ile Thr Gly Glu Gly Glu Gly Lys
20          25          30
Pro Phe Gln Gly Thr His Thr Ile Thr Leu Lys Val Lys Glu Gly Gly
35          40          45
Pro Leu Pro Phe Pro Tyr Asp Ile Leu Thr Thr Ala Phe Gln Tyr Gly
50          55          60
Asn Arg Val Phe Thr Lys Tyr Pro Arg Asp Ile Pro Asp Tyr Phe Lys
65          70          75          80

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Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Phe Glu  
85 90 95

Asp Gln Gly Ile Cys Thr Val Thr Ser Asp Ile Lys Leu Glu Gly Asp  
100 105 110

Cys Phe Phe Tyr Glu Ile Arg Phe Tyr Gly Val Asn Phe Pro Ser Asn  
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu  
130 135 140

Asn Met Tyr Val Arg Asp Gly Val Leu Leu Gly Asp Val Asn Arg Thr  
145 150 155 160

Leu Leu Leu Glu Gly Asp Lys His His Arg Cys Asn Phe Arg Ser Thr  
165 170 175

Tyr Arg Ala Lys Lys Gly Val Val Leu Pro Glu Tyr His Phe Val Asp  
180 185 190

His Arg Ile Glu Ile Leu Ser His Asp Lys Asp Tyr Asn Thr Val Glu  
195 200 205

Val Tyr Glu Asn Ala Val Ala Arg Pro Ser Met Leu Pro Ser Lys Ala  
210 215 220

Lys Glu Ser Ala His His His His His His  
225 230

<210> 16

<211> 234

<212> PRT

<213> Montastraea cavernosa

<400> 16

Met Ser Val Ile Lys Pro Asp Met Lys Ile Lys Leu Arg Met Glu Gly  
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Ala Val Asn Gly His Asn Phe Val Ile Glu Gly Glu Gly Lys Gly Lys  
20 25 30

Pro Phe Glu Gly Thr Gln Thr Ile Asn Leu Thr Val Lys Glu Gly Gly  
35 40 45

Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Ala Ala Phe Gln Tyr Gly  
50 55 60

Asn Arg Ala Phe Thr Lys Tyr Pro Arg Asp Ile Ala Asp Tyr Phe Lys  
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Tyr Glu  
85 90 95

Asp Gln Gly Ile Cys Ile Ile Lys Ser Asp Ile Arg Met Glu Gly Asp  
100 105 110

Cys Phe Ile Tyr Glu Ile Arg Tyr Asp Gly Val Asn Phe Pro Pro Ser  
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu  
130 135 140

Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala  
145 150 155 160

Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr  
165 170 175

Tyr Lys Ala Lys Lys Arg Val Gln Leu Pro Asp Tyr His Phe Val Asp  
180 185 190

His Arg Ile Glu Ile Leu Ser His Asp Asn Asp Tyr Asn Thr Val Lys  
195 200 205

Leu Ser Glu Asn Ala Glu Ala Arg Tyr Ser Met Leu Pro Ser Gln Ala  
210 215 220

Lys Glu Ser Ala His His His His His His  
225 230

<210> 17

<211> 234

<212> PRT

<213> Montastraea cavernosa

<400> 17

Met Ser Val Ile Lys Pro Asp Met Lys Ile Lys Leu Arg Met Gln Gly  
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Val Val Asn Gly His Lys Phe Val Ile Lys Gly Glu Gly Glu Gly Lys  
20 25 30

Pro Phe Glu Gly Thr Gln Thr Ile Asn Leu Thr Val Lys Glu Gly Ala  
35 40 45

Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Ser Ala Phe Gln Tyr Gly  
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Phe Lys  
65 70 75 80

Gln Thr Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ile Met Ala Tyr Glu  
85 90 95

Asp Gln Ser Ile Cys Thr Ala Thr Ser Asp Ile Lys Met Glu Gly Asp  
100 105 110

Cys Phe Ile Tyr Glu Ile Gln Phe His Gly Val Asn Phe Pro Pro Asn  
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu  
130 135 140



Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala  
 145 150 155 160

Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr  
 165 170 175

Tyr Lys Ala Lys Lys Asp Val His Leu Pro Asp Tyr His Tyr Val Asp  
 180 185 190

His Arg Ile Glu Ile Leu Ser His Asp Lys Asp Tyr Lys Asn Val Thr  
 195 200 205

Leu Tyr Glu His Ala Lys Ala Arg Tyr Ser Met Leu Pro Ser Lys Ala  
 210 215 220

Lys Glu Ser Ala His His His His His His  
 225 230

<210> 18  
 <211> 238  
 <212> PRT  
 <213> Scolymia cubensis

<400> 18

Met Ser Ala Ile Lys Thr Val Val Lys Gln Phe Met Lys Ile Lys Met  
 1 5 10 15

Ser Leu Glu Gly Thr Val Asn Gly His Tyr Phe Lys Ile Val Gly Glu  
 20 25 30

Gly Asp Gly Thr Pro Phe Glu Gly Lys Gln Thr Leu His Leu Lys Val  
 35 40 45

Lys Glu Gly Ala Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Thr Ala  
 50 55 60

Leu His Tyr Gly Asn Arg Val Phe Val Glu Tyr Pro Glu Asn Ile Pro  
 65 70 75 80

Asp Tyr Phe Lys Gln Ser Phe Pro Lys Gly Tyr Ser Trp Glu Arg Ser  
 85 90 95

Leu Thr Phe Glu Asp Gly Gly Ile Cys Ile Ala Arg Ser Asp Ile Lys  
 100 105 110

Met Val Gly Asp Thr Phe His Asn Glu Val Gln Phe Tyr Gly Val Asn  
 115 120 125

Phe Pro Pro Asn Gly Pro Val Met Gln Arg His Thr Val Lys Trp Glu  
 130 135 140

Pro Ser Thr Glu Lys Ile Tyr Val Arg Asp Gly Val Leu Thr Gly Asp  
 145 150 155 160

Ile Thr Met Ala Leu Leu Lys Gly Gly Thr His Tyr Arg Cys Asp  
 165 170 175

Phe Arg Thr Thr Tyr Lys Ala Lys Glu Lys Gly Pro Lys Phe Pro Gly  
                   180                                  185                                  190  
 Tyr His Leu Val Asp His Cys Ile Glu Ile Thr Ser His Asp Lys Asp  
                   195                                  200                                  205  
 Tyr Asn Val Val Glu Leu Tyr Glu His Ala Val Ala His Ser Gly Leu  
                   210                                  215                                  220  
 Pro Asp Ser Ala Asn Arg Gln Ser His His His His His His His  
                   225                                  230                                  235

<210> 19  
 <211> 765  
 <212> DNA  
 <213> Acropora aculeus

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 catgaattca cgatcgaagg tgtaggaact gggtagcctt acgaaggga acagatgtcc 180  
 gaattagtga tcatcaagcc taagggaag ccccttccat tctcctttga catactgtca 240  
 tcagtctttc aatatggaaa caggtgcttc acaaagtacc ctgcagacat gcctgactat 300  
 ttcaagcaag cattcccaga tggaatgtca tatgaaaggc cttttctatt tgaggatgga 360  
 gcagttgcta cagccagctg gaacattcgt ctgaaggaa attgcttcat ccacaattcc 420  
 atctttcatg gcgtaaactt tcccgatgat ggaccgtaa tgaaaaagaa gacaattggc 480  
 tgggataagt ccttcgaaaa aatgactgtg tctaaagagg tgttaagagg tgatgtgact 540  
 atgtttctta tgctcgaagg aggtgggtac cacagatgcc agtttcactc cacttacaaa 600  
 acagagaagc cggtcgaact gccccgaat catgtcgtag aacatcaaatt tgtgaggacc 660  
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<210> 20  
 <211> 765  
 <212> DNA  
 <213> Acropora aculeus

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 catgaattcg agatcgaagg tgtaggaact ggacaccctt acgaaggga acacatggcc 180  
 gaattagtga tcataaagcc tgcgggaaaa ccccttccat tctcctttga catactgtca 240

acagtcattc aatacggaaa cagatgcttc actaagtacc ctgcagacct gcctgactat 300  
 ttcaagcaag catacccagg tggaatgtca tatgaaaggt catttgtgta tcaggatgga 360  
 ggaattgcta cagcgagctg gaacgttagt ctcgagggaa attgcttcat ccacaaatcc 420  
 acctatcttg gtgtaaactt tcctgctgat ggacccgtaa tgacaaagaa gacaattggc 480  
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 ccagagaagc cggctcgaact gccccgaat catgtcatag aacatcacat tgtgaggacc 660  
 gaccttggca agactgcaaa aggcttcatg gtcaagctgg tacaacatgc tgcggtcat 720  
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<210> 21  
 <211> 716  
 <212> DNA  
 <213> Acropora aculeus

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 aagcagatgt ccgaattagt gatcgtcaag cctaagggaa agccccctcc attctccttt 180  
 gacatactgt catcagtctt tcaatatgga aacaggtgct tcacaaagta ccctgcagac 240  
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 attgtgagga ccgaccttgg ccaaagtga aaaggcttca cagtcaagct ggaagcacat 660  
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<210> 22  
 <211> 765  
 <212> DNA  
 <213> Acropora aculeus

<400> 22  
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gaattagtga tcataaagcc tgcgggaaaa ccccttccat tctcctttga catactgtca 240  
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ggaattgcta cagcgagctg gaacgtcggt ctcgaggga attgcttcat ccacaaatcc 420  
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<210> 23

<211> 765

<212> DNA

<213> *Acropora aculeus*

<400> 23

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catgaattcg agatcgaagg tgtaggaact ggacaccctt acgaagggaac acacatggcc 180  
gaattagtga tcataaagcc tgcgggaaaa ccccttccat tctcctttga catactgtca 240  
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ggaattgcta cagcgagctg gaacgttggt ctcgaggga attgcttcat ccacaaatcc 420  
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<210> 24

<211> 711  
<212> DNA  
<213> Acropora aculeus

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ccttacgagg gggagcagac ggtgaagctc actgtcacca agggaggacc tctgccattt 180  
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tgtttcatct acaatgtcaa gttctctggt ttgaactttc ctccaatgg accggttatg 420  
cagaagaaga cacagggctg ggaacccaac actgagcgtc tctttgcacg agatggaatg 480  
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ttcaaactta cttacaaggc aaagaagcct gtgaggatgc cagggtatca ctatgttgac 600  
cgcaaactgg atgtaaccaa tcacaacagg gattacactt ccgttgagca gcgtgaaatt 660  
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<210> 25  
<211> 735  
<212> DNA  
<213> Acropora aculeus

<400> 25  
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gtcgaaggcg atggaaaagg aaagccttac gagggggagc agacggtgaa gctcactgtc 180  
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atgccagggt atcactatgt tgaccgcaaa ctggatgtaa ccaatcacia cagggttac 660  
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caccatcact aataa

735

&lt;210&gt; 26

&lt;211&gt; 711

&lt;212&gt; DNA

&lt;213&gt; Acropora hyacinthus

&lt;400&gt; 26

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ccttacgagg gggagcaaac ggtaaggctg actgtcacca agggcggacc tctgccgttt 180  
gcttgggata ttttatcacc acagtacacag tacggaagca taccattcac caagtaccct 240  
gaagacatcc ctgactatgt gaagcagtca ttcccgagg gatatacatg ggagaggatc 300  
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tgtttcatct accatgtcaa gttctctggt ttgaactttc ctcccaatgg acctgttatg 420  
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cgcaaaactgg atgtaaccaa tcacaacaag gattacactt ctgttgagca gcgtgaaatt 660  
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&lt;210&gt; 27

&lt;211&gt; 741

&lt;212&gt; DNA

&lt;213&gt; Acropora millepora

&lt;400&gt; 27

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ggaactggaa acccttacga aggcacacag atgtccgaat tagtgatcac cgagcctgca 180  
ggaaaacccc ttccattctc ctttgacatt ctgtcaacag tctttcagta tggaaacagg 240  
tgcttcacaa agtaccctga aggaatgact gactattttca agcaagcatt ccagatgga 300  
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attcgtcttg agagagattg cttcatccac aaatccatct atcatggcgt taactttccc 420  
gctgatggac ccgtaatgaa aaagaagacc attggctggg ataaagcctt cgaaaaaatg 480  
actgtgtcca aagacgtttt aagaggatgat gtgactgagt ttcttatgct cgaaggaggt 540

ggttaccaca gctgccagtt tcaactccact tacaaccag agaagccggt tacactgccc 600  
 cctaatacatg tcgtggaaca tcacattgtg aggactgacc ttggccaaac tgcaaaaggc 660  
 ttcacagtca agctggaaga acatgctgcg gctcatgtta accctttgaa ggttcaccat 720  
 caccatcacc atcactaata a 741

<210> 28  
 <211> 726  
 <212> DNA  
 <213> *Acropora millepora*

<400> 28  
 ttgattgatt gaaggagaaa tatcatgaag ccatctatcc tcaacatgtc ttattcaaag 60  
 caaggcatcg tacaagaaat gaagacgaaa taccatatgg aaggcagtgt caatggccat 120  
 gaattcacga tcgaagggtgt aggaactggg tacccttacg aagggaaaca gatatccgaa 180  
 ttagtgatca tcaagcctgc gggaaaaccc cttccattct cctttgacat actgtcatca 240  
 gtctttcaat atggaaacag gtgcttcaca aagtaccctg cagacatgcc tgactatttc 300  
 aagcaagcat tcccagatgg aatgtcatat gaaaggatcat ttctatttga ggatggagca 360  
 gttgccacag ccagctggaa cattcgtctc gaaggaaatt gcttcatcca caaatccatc 420  
 tttcatggcg taaactttcc cgctgatgga cccgtaatga aaaagaagac aattgactgg 480  
 gataagtcct tcgaaaaaat gactgtgtct aaagagggtgc taagagggtga cgtgactatg 540  
 tttcttatgc tcgaaggagg tggttctcac agatgccaat ttactccac ttacaaaaca 600  
 gagaagccgg tcacactgcc cccgaatcat gtcgtagaac atcaaattgt gaggaccgac 660  
 cttggccaaa ctgcaaaagg cttcacagtc aagctggaag aacatgctgc ggctcatgtt 720  
 agccta 726

<210> 29  
 <211> 761  
 <212> DNA  
 <213> *Acropora millepora*

<400> 29  
 ttgattgatt gaaggagaaa tatcatgaag ccatctatcc tcaacatgtc ttattcaaag 60  
 caaggcatcg tacaagaaat gaagacgaaa taccatatgg aaggcagtgt caatggccat 120  
 gaattcacga tcgaagggtgt aggaactggg tacccttacg aagggaaaca gatgtccgaa 180  
 ttagtgatca tcaagcctgc gggaaaaccc cttccattct cctttgacat actgtcatca 240  
 gtctttcaat atggaaacag gtgcttcaca aagtaccctg cagacatgcc tgactatttc 300

aagcaagcat tcccagatgg aatgtcatat gaaaggtcat ttctatttga ggatggagca 360  
 gttgccacag ccagctggaa cattcgtctc gaaggaaatt gcttcatcca caaatccatc 420  
 tttcatggcg taaactttcc cgctgatgga cccgtaatga aaaagaagac aattgactgg 480  
 gataagtcct tcgaaaaaat gactgtgtct aaagaggtgc taagaggtga cgtgactatg 540  
 tttcttatgc tcgaaggagg tggttctcac agatgccaat ttcaactccac ttacaaaaca 600  
 gagaagccgg tcacactgcc cccgaatcat gtcgtagaac atcaaattgt gaggaccgac 660  
 cttggccaaa ctgcaaaagg cttcacagtc aagctggaag aacatgctgc ggctcatgta 720  
 accctttgaa ggttcaacat caccatcacc atcactaata a 761

<210> 30

<211> 762

<212> DNA

<213> Acropora millepora

<400> 30

ttgattgatt gaaggagaaa tatcatgagg caatctatcc tcaacatgtc ttattcaaag 60  
 cagggcatcg tacaagaaat gaagacgaaa taccgtatgg aaggcagtgt caatggccat 120  
 gaattcacga tcgaagggtg aggaactggg tacccttacg aagggaagca gatgtccgaa 180  
 ttagtgatcg tcaagcctaa gggaaagccc cttccattct cctttgacat actgtcatca 240  
 gtctttcaat atggaaacag gtgcttcaca aagtaccctg cagacatgcc tgactatttc 300  
 aagcaagcat tcccagatgg aatgtcatat gaaaggtcat ttctatttga ggatggagca 360  
 gttgctacag ccagctggaa cattcgtctc gaaggaaatt gcttcatcca caattccatc 420  
 tttcatggcg taaactttcc cgctgatgga cccgtaatga aaaagaagac aattggctgg 480  
 gataagtcct tcgaaaaaat gactgtgtct aaagaggtgt taagaggtga tgtgactatg 540  
 tttcttatgc tcgaaggagg tggttaccac agatgccagt ttcaactccac ttacaaaaca 600  
 gtgaagccgg tcgaactgcc cccgaatcat gtcgtagaac atcaaattgt gaggaccgac 660  
 cttggccaaa gtgcaaaagg cttcacagtc aagctggaag cacatgctgc ggctcatgtt 720  
 aaccctttga aggttcaaca tcaccatcac catcactaat aa 762

<210> 31

<211> 762

<212> DNA

<213> Acropora millepora

<400> 31

ttgattgatt gaaggagaaa tatcatgaag ccatctatcc tcaacatgtc tcattcaaag 60



caaggcatcg cacaagtaat gaagacgaaa taccatatgg aaggcagtgt caatggccat 120  
 gaattcacga tcgaagggtgt aggaactgga aacccttacg aaggctcaca gatgtccgag 180  
 ttagtgatca ccaagcctgc aggaaaaccc cttccattct cctttgacat tctctcaaca 240  
 gtctttcaat atggaaacag gtgcttcaca aagtaccctg aaggaatgac tgactatttc 300  
 aagcaagcat tcccagatgg aatgtcatat gaaagggtcat ttctatatga ggatggagga 360  
 gttgctacag ccagctggaa cattcgtctt gagagagggt gcttcatcca caaatccatc 420  
 tatcatggcg ttaactttcc cgctgatgga cccgtaatga aaaagaagac cattggctgg 480  
 gataaggcct tcgaaaaaat gactgtgtcc aaagacgtgt taagagggtga tgtgactggg 540  
 tttcttatgc tcgaaggagg tggttaccac aactgccagt ttactccac ttacaaacca 600  
 gaaaagccgg ttactactgcc cccgaatcat gtcgtggaac atcacattgt gaggactgac 660  
 cttggccaaa ctgcaaaagg cttcacagcc aagctggaag aacatgctgc ggctcatgta 720  
 aaccctttga aggttcaaca tcaccatcac catcactaat aa 762

<210> 32

<211> 741

<212> DNA

<213> Acropora millepora

<400> 32

ttgattgatt gaaggagaaa tatcatgtct tattcaaagc agggcatcgt acaagaaatg 60  
 aagacgaaat accatatgga aggcagtgtc aatggccatg aattcacgat cgaagggtga 120  
 ggaactgggt acccttacga agggaaacag atgtccgaat tagtgatcat caagcctgcg 180  
 ggaaaacccc ttccattctc ctttgacata ctgtcatcag tctttcaata tggaaacagg 240  
 tgcttcacaa agtaccctgc agacatgcct gactatttca agcaagcatt cccagatgga 300  
 atgtcatatg aaagggtcatt tctatttgag gatggagcag ttgctacagc cagctggaac 360  
 attcgtctcg aaggaaattg cttcatccac aaatccatct ttcatggcgt aaactttccc 420  
 gctgatggac ccgtaatgaa aaagaagaca attgactggg ataagtcctt cgaaaaaatg 480  
 actgtgtcta aagagggtgt aagagggtgac gtgactatgt ttcttatgct cgaaggaggt 540  
 ggttctcaca gatgccaatt tcactccact taaaaacag agaagccggt cacactgccc 600  
 ccgaatcatg tcgtagaaca tcaaattgtg aggaccgacc ttggccaaag tgcaaaaggc 660  
 ttacagtca agctggaagc acatgctgcg gctcatgtta accctttgaa ggttaaacad 720  
 caccatcacc atcactaata a 741

<210> 33

<211> 744  
 <212> DNA  
 <213> *Acropora millepora*

<400> 33  
 ttgattgatt gaaggagaaa tatcatggct ctgtcaaagc acggtttaac aaaggacatg 60  
 acgatgaaat accacatgga agggctctgtc gatgggcata aatttgtgat cacgggccac 120  
 ggcaatggaa atcctttcga agggaaacag actatgaatc tgtgtgtggt tgaaggggga 180  
 cccctgccat tctccgaaga cttttgtct gctacgtttg actacggaaa cagggctctc 240  
 actgaatatc ctcaaggcat ggttgacttt ttcaagaatt catgtccagc tggatacaca 300  
 tggcacaggt ctttactctt tgaagatgga gcagtttgca caactagtgc agatataaca 360  
 gtgagtgttg aggagaactg cttttatcac aattccaagt ttcattggagt gaactttcct 420  
 gctgatggac ctgtgatgaa aaagatgaca actaattggg agccatcctg cgagaaaatc 480  
 ataccagtac ctagacaggg gatattgaaa ggggatattg ccatgtacct cttctgaag 540  
 gatggtgggc gttatcgggtg ccagttcgac acaatttaca aagcaaagtc tgacccgaaa 600  
 gagatgccgg agtggcactt catccaacat aagctcaccg gggaagaccg cagcgatgct 660  
 aagaaccaga aatggcaact ggtagaacat gctgttgctt cccgatccgc attgcccgga 720  
 catcaccatc accatcacta ataa 744

<210> 34  
 <211> 711  
 <212> DNA  
 <213> *Acropora millepora*

<400> 34  
 ttgattgatt gaaggagaaa tatcatgagt gtgatcgcta aacaaatgac ctacaaggtt 60  
 tatatgtcag gcacgggtcaa tggacactac tttgaggtcg aaggcgatgg aaaaggtaag 120  
 ccctacgagg gggagcagac ggtaaagctc actgtcacca agggcggacc tctgccattt 180  
 gcttgggata ttttatcacc acagtgtcag tacggaagca taccattcac caagtaccct 240  
 gaagacatcc ctgactatgt aaagcagtca ttcccggagg gctatacatg ggagaggatc 300  
 atgaactttg aagatgggtgc agtgtgtact gtcagcaatg attccagcat ccaaggcaac 360  
 tgtttcatct accatgtcaa gttctctgggt ttgaactttc ctcccaatgg acctgtcatg 420  
 cagaagaaga cacagggctg ggaacccaac actgagcgtc tctttgcacg agatggaatg 480  
 ctgctaggaa acaactttat ggctctgaag ttagaaggag gcggtcacta tttgtgtgaa 540  
 ttcaaaaacta cttacaaggc aaagaagcct gtgaagatgc cagggtatca ctatgttgac 600  
 cgaaaactgg atgtaaccaa tcacaacaag gattacactt cggttgagca gtgtgaaatt 660

tccattgcac gcaaacctgt ggtcgcccat caccatcacc atcactaata a 711

<210> 35  
<211> 765  
<212> DNA  
<213> *Acropora nobilis*

<400> 35  
ttgattgatt gaaggagaaa tatcatgatc aagccatcta tcctcaacat gtcttattca 60  
aagcaaggca tcgcacaagt aatgaagacg aaataccata tggaaggcag tgtcaatggc 120  
catgaattca cgatcgaagg tgtaggaact ggaaaccctt acgaaggcac acagatgtcc 180  
gaattagtga tcaccaagcc tgcaggaaaa ccccttccat tctcctttga cattctgtca 240  
acagtctttc aatatggaaa caggtgcttc acaaagtacc ctgaaggaat gactgactat 300  
ttcaagcaag cattcccaga tggaatgtca tgtgaaaggt catttctata tgaggatgga 360  
ggagttgcta cagccagctg gaacattcgt cttgagagag attgcttcat ccacaaatcc 420  
atctatcatg gcgttaactt tcccgtgat ggacccgtaa tgaaaaagaa gaccattggc 480  
tgggataaag ctttcgaaaa aatgactgtg tccaaagacg tgtaagagg tgatgtgact 540  
gagtttctta tgctcgaagg aggtggttac cacagctgcc agtttcactc cacttacaaa 600  
ccagaaaagc cggctgcact gccccgaat catgtcgtag aacatcacat tgtgaggact 660  
gaccttggcc aaagtgc aaaaggcttcaca gtcaagctgg aagaacatgc tgcggctcat 720  
gttaaccctt tgaaggttca acatcaccat caccatcact aataa 765

<210> 36  
<211> 741  
<212> DNA  
<213> *Acropora nobilis*

<400> 36  
ttgattgatt gaaggagaaa tatcatgtct tattcaaagc agggcatcgc acaagtaatg 60  
aagacgaaat accatatgga aggcagtgtc aatggccatg aattcacgat cgaagggtga 120  
ggaactggaa acccttacga aggcacacag atgtccgaat tggatgatcac caagcctgca 180  
ggaaaacccc ttccattctc ctttgacatt ctgtcaacag tctttcaata tggaaacagg 240  
tgcttcacaa agtaccctga aggaatgact gactatttca agcaagcatt ccagatgga 300  
atgtcatatg aaaggtcatt tctatatgag gatggaggag ttgctacagc cggttggaac 360  
attcgtcttg agagagattg cttcatccac aaatccatct atcatggcgt taactttccc 420  
gctgatggac ccgtaatgaa gaagaagacc attggctggg ataaagcctt cgaaaaaatg 480

actgtgtcca aagacgtggt aagaggtgat gtgactgggt ttcttatgct cgaaggaggt 540  
 gggtaccaca gctgccagtt tcaactccact taaaaaccag aaaagccggc tgcactgccc 600  
 ccgaatcatg tcgtagaaca tcacattgtg aggactgacc ttggccaaag tgcaaaaggc 660  
 ttacacagtca agctggaaga acatgctgcg gctcatgtta accctttgaa gggtcaacat 720  
 caccatcacc atcactaata a 741

<210> 37  
 <211> 741  
 <212> DNA  
 <213> *Acropora nobilis*

<400> 37  
 ttgattgatt gaaggagaaa tatcatgtct tattcaaagc agggcatcgc acaagaaatg 60  
 aagacgaaat accatatgga aggcagtgtc aatggccatg aattcacggc cgaagggtgta 120  
 gggactgggt acccttacga aggggaacag atgtccgaat tagtgatcat cgagcctgcg 180  
 ggaaaacccc ttccattctc ctttgacata ctgtcatcag tctttcagta tggaaacagg 240  
 tgcttcacaa aataccctgc agacatgcct gactatttca agcaagcatt tccagatgga 300  
 atgtcatatg aaaggtcatt tctatttgag gatggagcag ttgctacagc cagctggaaa 360  
 attcgtctcg aaggaaattg cttcatccac aactccatct ttaatggcgt aaactttccc 420  
 gctgatggac ccgtaatgga aaagaagaca attggctggg ataagtcctt cgaaaaaatg 480  
 actgtgtcta aagaggtgct aagaggtgat gtgactatgt ttcttatgct cgaaggaggt 540  
 gggtctcaca gatgccagtt tcaactccact taaaaacag agaagccggc cactactgccc 600  
 ccgaatcatg tcgtagaaca tcaaattgtg aggaccgacc ttggccaaag tgcaaaaggc 660  
 ttacacagtca agctggaagc acatgctgcg gctcatgtta accctttgaa gggttaacat 720  
 caccatcacc atcactaata a 741

<210> 38  
 <211> 726  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 38  
 ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60  
 cacatggaag gtactgttaa cgggcacgcc cttacaattg aaggcaaagg aaaaggcgat 120  
 cttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180  
 tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcgc gaagtatcca 240  
 gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360  
 gagatgtggt ttatctatga cgtccgtttt cacgggtgtga actttcccg ccaatggccca 420  
 atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480  
 ggggtgctga aggggtgatgt taatatgact ctctgtgttg aaggaggtgg ccattaccga 540  
 gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600  
 atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660  
 gaggcagcag ttgctcgtca ttctccgctg cctaagggtg ctcatcacca tcaccatcac 720  
 taataa 726

<210> 39  
 <211> 725  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 39  
 ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60  
 cacatggaag gtactgttaa cgggcacgcc ttacaattg aaggcaaagg aaaaggcgat 120  
 ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180  
 tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240  
 gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300  
 attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360  
 gagatgtggt ttatctatga cgtccgtttt cacgggtgtga actttcccg ccaatggccca 420  
 atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480  
 ggggtgctga aggggtgatgt taatatgact ctctgtgttg aaggaggtgg ccattaccga 540  
 gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600  
 atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660  
 gaggcagcag ttgctcgtca ttctccgctg cctaagggtg ctcatcacca tcacatcact 720  
 aataa 725

<210> 40  
 <211> 726  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 40  
 ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60

cacatggaag gtactgttaa cgggcacgcc ttacaattg aaggcaaagg aaaaggcgat 120  
 ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc ttgcccgttc 180  
 tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtggtcac gaagtatcca 240  
 gaagacatac cagacttttt caagcagggtg tttcctgaag ggtaccactg ggaaagaagt 300  
 attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360  
 gagatgtggt ttatctatga cgtccgtttt cacggtgtga actttcccgc caatggccca 420  
 atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480  
 ggggtgctga agggatgatg taatatgact ctctgtgttg aaggaggtgg ccattaccga 540  
 gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600  
 atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660  
 ggggcagcag ttgctcgtca ttctccgctg cctaagggtt ctcatcacca tcaccatcac 720  
 taataa 726

<210> 41  
 <211> 725  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 41  
 ttgattgatt gaaggaaaat atcatgagtg tgattgtaaa ggaaatgatg actaagctac 60  
 acatggaagg tactgttaac gggcacgcct ttacaattga aggcaaagga aaaggcgatc 120  
 cttacaatgg agtgcagtc atgaaccttg acgtcaaagg cgggtgcgcct ttgcccgttct 180  
 ctttcgatct cttgacgcc gcattcatgt acggcaacag agtggttcacg aagtatccag 240  
 aagacatacc agactttttc aagcagggtg ttctgaagg gtaccactgg gaaagaagta 300  
 ttacctttga agatcaggcc gtttgtacgg caaccagcca cataaggctg gaccagaaag 360  
 agatgtgttt tatctatgac gtccgttttc acggtgtgaa ctttcccgc aatggcccaa 420  
 tcatgcagaa gaagatactg ggatgggagc catccactga gaaaatgtat gcacgtgatg 480  
 ggggtgctgaa gggatgatgt aatgtgactc ttctgtgtga aggaggtggc cattaccgag 540  
 ctgacttcag aactacttac aaagcaaaga agccagtcaa cctgccaggc tatcacttca 600  
 tagaccaccg cattgagatt accaagcaca gcaaagatta caccaatgtt gctttgtatg 660  
 aggcagcagt tgctcgtcat tctccgctgc ctaagggtgc tcatcaccat caccatcact 720  
 aataa 725

<210> 42

<211> 726  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 42  
 ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60  
 cacatggaag gtactgttaa cgggcacgcc tttaacaattg aaggcaaagg agagggcgat 120  
 ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180  
 tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240  
 gaagacatac cagacttttt caagcagggtg tttcctgaag ggtaccactg ggaaagaagt 300  
 attacctttg aagatcaggc cgtttgtacg gctaccagcc acataaggct ggaccagaaa 360  
 gagatgtggt ttatctatga cgtccgtttt cacggtgtga actttccgc caatggccca 420  
 atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480  
 ggggtgctga agggatgatgt taatatgact ctctgtgttg aaggaggtgg ccattaccga 540  
 gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600  
 atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660  
 ggggcagcag ttgctogtca ttctccgctg cctaagggtg ctcacacca tcaccatcac 720  
 taataa 726

<210> 43  
 <211> 726  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 43  
 ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60  
 cacatggaag gtactgttaa cgggcacgcc tttaacaattg aaggcaaagg aaaaggcgat 120  
 ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180  
 tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240  
 gaagacatac cagacttttt caagcagggtg tttcctgaag ggtaccactg ggaaagaagt 300  
 attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360  
 gagatgtggt ttatctatga cgtccgtttt cacggtgtga actttccgc caatggccca 420  
 atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480  
 ggggtgctga agggatgatgt taatatgact ctctgtgttg aaggaggtgg ccattaccga 540  
 gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600  
 atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

gaggcagcag ttgctcgtca ttctccgctg cctaagggtg ctcacacca tcaccatcac 720

taataa 726

<210> 44

<211> 726

<212> DNA

<213> Agaricia fragilis

<400> 44

ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60

cacatggaag gtactgttaa cgggcacgcc tttaacaattg aaggcaaagg aaaaggcgat 120

ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc ttgcccgttc 180

tctttcgate tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240

gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360

gagatgtggt ttatctatga cgtccgtttt cacggtgtga actttccgc caatggccca 420

atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480

ggggtgctga aggggtgatgt taatacgact ctctgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

gaggcagcag ttgctcgtca ttctccgctg cctaagggtg ctcacacca tcaccatcac 720

taataa 726

<210> 45

<211> 237

<212> PRT

<213> Acropora aculeus

<400> 45

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr  
1 5 10 15

Arg Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile  
35 40 45

Ile Lys Pro Lys Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
50 55 60



Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn  
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Asn Ser Ile Phe His Gly  
115 120 125

Val Asn Phe Pro Asp Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly  
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg  
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Tyr His Arg  
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Glu Leu Pro  
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln  
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His  
210 215 220

Val Asn Pro Leu Lys Val Gln Gln His His His His His  
225 230 235

<210> 46

<211> 237

<212> PRT

<213> Acropora aculeus

<400> 46

Met Ser Leu Ser Lys His Gly Ile Thr Gln Glu Met Pro Thr Lys Tyr  
1 5 10 15

His Met Lys Gly Ser Val Asn Gly His Glu Phe Glu Ile Glu Gly Val  
20 25 30

Gly Thr Gly His Pro Tyr Glu Gly Thr His Met Ala Glu Leu Val Ile  
35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
50 55 60

Thr Val Ile Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
65 70 75 80

Leu Pro Asp Tyr Phe Lys Gln Ala Tyr Pro Gly Gly Met Ser Tyr Glu  
85 90 95

Arg Ser Phe Val Tyr Gln Asp Gly Gly Ile Ala Thr Ala Ser Trp Asn  
100 105 110

Val Ser Leu Glu Gly Asn Cys Phe Ile His Lys Ser Thr Tyr Leu Gly  
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Thr Lys Lys Thr Ile Gly  
130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Gly Phe Asn Glu Val Leu Arg  
145 150 155 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser  
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Glu Leu Pro  
180 185 190

Pro Asn His Val Ile Glu His His Ile Val Arg Thr Asp Leu Gly Lys  
195 200 205

Thr Ala Lys Gly Phe Met Val Lys Leu Val Gln His Ala Ala Ala His  
210 215 220

Val Asn Thr Leu Lys Val Gln His His His His His His  
225 230 235

<210> 47

<211> 265

<212> PRT

<213> Acropora aculeus

<400> 47

Met Thr Met Ile Thr Pro Ser Tyr Leu Gly Asp Thr Ile Glu Tyr Ser  
1 5 10 15

Ser Tyr Ala Ser Asn Ala Leu Gly Ala Leu Pro Tyr Gly Arg Pro Ala  
20 25 30

Gly Gly Arg Thr Ser Asp Leu Ser Tyr Ser Lys Gln Gly Ile Val Gln  
35 40 45

Glu Met Lys Thr Lys Tyr Arg Met Glu Gly Ser Val Asn Gly His Glu  
50 55 60

Phe Thr Ile Glu Gly Val Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln  
65 70 75 80

Met Ser Glu Leu Val Ile Val Lys Pro Lys Gly Lys Pro Leu Pro Phe  
85 90 95

Ser Phe Asp Ile Leu Ser Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe  
100 105 110

Thr Lys Tyr Pro Ala Asp Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro  
115 120 125

Asp Gly Met Ser Tyr Glu Arg Ser Phe Leu Phe Glu Asp Gly Ala Val  
130 135 140

Ala Thr Ala Ser Trp Asn Ile Arg Leu Glu Gly Asn Cys Phe Ile His  
145 150 155 160

Asn Ser Ile Phe His Gly Val Asn Phe Pro Ala Asp Gly Pro Val Met  
165 170 175

Lys Lys Lys Thr Ile Gly Trp Asp Lys Ser Phe Glu Lys Met Thr Val  
180 185 190

Ser Lys Glu Val Leu Arg Gly Asp Val Thr Met Phe Leu Met Leu Glu  
195 200 205

Gly Gly Gly Tyr His Arg Cys Gln Phe His Ser Thr Tyr Lys Thr Val  
210 215 220

Lys Pro Val Glu Leu Pro Pro Asn His Val Val Glu His Gln Ile Val  
225 230 235 240

Arg Thr Asp Leu Gly Gln Ser Ala Lys Gly Phe Thr Val Lys Leu Glu  
245 250 255

Ala His Ala Ala Ala His Val Thr Leu  
260 265

<210> 48

<211> 237

<212> PRT

<213> Acropora aculeus

<400> 48

Met Ser Leu Ser Lys His Gly Ile Thr Gln Glu Met Pro Thr Lys Tyr  
1 5 10 15

His Met Lys Gly Ser Val Asn Gly His Glu Phe Glu Ile Glu Gly Val  
20 25 30

Gly Thr Gly His Pro Tyr Glu Gly Thr His Met Ala Glu Leu Val Ile  
35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
50 55 60

Thr Val Ile Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
65 70 75 80

Leu Pro Asp Tyr Phe Lys Gln Ala Tyr Pro Gly Gly Met Ser Tyr Glu  
85 90 95

Arg Ser Phe Val Phe Gln Asp Gly Gly Ile Ala Thr Ala Ser Trp Asn  
100 105 110

Val Gly Leu Glu Gly Asn Cys Phe Ile His Lys Ser Thr Tyr Leu Gly  
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Thr Lys Lys Thr Ile Gly  
 130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Gly Phe Asn Glu Val Leu Arg  
 145 150 155 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser  
 165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Lys Leu Pro  
 180 185 190

Pro Asn His Val Ile Glu His His Ile Val Arg Thr Asp Leu Gly Lys  
 195 200 205

Thr Ala Lys Gly Phe Met Val Lys Leu Val Gln His Ala Ala Ala His  
 210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His  
 225 230 235

<210> 49  
 <211> 237  
 <212> PRT  
 <213> Acropora aculeus

<400> 49

Met Ser Leu Ser Lys His Gly Ile Thr Gln Glu Met Pro Thr Lys Tyr  
 1 5 10 15

His Met Lys Gly Asn Val Asn Gly His Glu Phe Glu Ile Glu Gly Val  
 20 25 30

Gly Thr Gly His Pro Tyr Glu Gly Thr His Met Ala Glu Leu Val Ile  
 35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
 50 55 60

Thr Val Ile Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
 65 70 75 80

Leu Pro Asp Tyr Phe Lys Gln Ala Tyr Pro Gly Gly Met Ser Tyr Glu  
 85 90 95

Arg Ser Phe Val Phe Gln Asp Gly Gly Ile Ala Thr Ala Ser Trp Asn  
 100 105 110

Val Gly Leu Glu Gly Asn Cys Phe Ile His Lys Ser Thr Tyr Leu Gly  
 115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Thr Lys Lys Thr Ile Gly  
 130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Gly Phe Asn Glu Val Leu Arg  
 145 150 155 160

Gly Asp Val Thr Gly Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser  
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Lys Leu Pro  
180 185 190

Pro Asn His Val Ile Glu His His Ile Val Arg Thr Asp Leu Gly Lys  
195 200 205

Thr Ala Lys Gly Phe Met Val Lys Leu Val Gln His Ala Ala Ala His  
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His  
225 230 235

<210> 50

<211> 227

<212> PRT

<213> Acropora aculeus

<400> 50

Met Ser Val Ile Ala Lys Gln Met Thr Tyr Lys Val Tyr Met Ser Gly  
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys  
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Lys Leu Thr Val Thr Lys Gly Gly  
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Ser Gln Tyr Gly  
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Val Lys  
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu  
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn  
100 105 110

Cys Phe Ile Tyr Asn Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn  
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Gln Gly Trp Glu Pro Asn Thr Glu  
130 135 140

Arg Leu Phe Ala Arg Asp Gly Met Leu Ile Gly Asn Asn Phe Met Ala  
145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Ser Thr  
165 170 175

Tyr Lys Ala Lys Lys Pro Val Arg Met Pro Gly Tyr His Tyr Val Asp  
180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Arg Asp Tyr Thr Ser Val Glu  
 195 200 205

Gln Arg Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His  
 210 215 220

His His His  
 225

<210> 51

<211> 227

<212> PRT

<213> Acropora aculeus

<400> 51

Met Ser Val Ile Ala Lys Gln Met Thr Tyr Lys Val Tyr Met Ser Gly  
 1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys  
 20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Lys Leu Thr Val Thr Lys Gly Gly  
 35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Ser Gln Tyr Gly  
 50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Val Lys  
 65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu  
 85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn  
 100 105 110

Cys Phe Ile Tyr Asn Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn  
 115 120 125

Gly Pro Val Met Arg Lys Lys Thr Arg Gly Trp Glu Pro Asn Thr Glu  
 130 135 140

Arg Leu Phe Ala Arg Asp Gly Met Leu Ile Gly Asn Asn Phe Met Ala  
 145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Ser Thr  
 165 170 175

Tyr Lys Ala Lys Lys Pro Val Arg Met Pro Gly Tyr His Tyr Val Asp  
 180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Arg Asp Tyr Thr Ser Val Glu  
 195 200 205

Gln Cys Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His  
 210 215 220

His His His  
225

<210> 52  
<211> 227  
<212> PRT  
<213> Acropora hyacinthus  
  
<400> 52

Met Ser Val Ile Ala Thr Gln Met Thr Tyr Lys Val Tyr Met Ser Gly  
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys  
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Arg Leu Thr Val Thr Lys Gly Gly  
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Ser Gln Tyr Gly  
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Tyr Val Lys  
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu  
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn  
100 105 110

Cys Phe Ile Tyr His Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn  
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Gln Gly Trp Glu Pro Asn Thr Glu  
130 135 140

Arg Leu Phe Ala Arg Asp Gly Val Leu Ile Gly Asn Asn Phe Met Ala  
145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Ser Thr  
165 170 175

Tyr Lys Ala Lys Lys Pro Val Lys Met Pro Gly Tyr His Phe Val Asp  
180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Lys Asp Tyr Thr Ser Val Glu  
195 200 205

Gln Arg Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His  
210 215 220

His His His  
225

<210> 53  
<211> 237

&lt;212&gt; PRT

&lt;213&gt; Acropora millepora

&lt;400&gt; 53

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr  
 1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
 20 25 30

Gly Thr Gly Asn Pro Tyr Glu Gly Thr Gln Met Ser Glu Leu Val Ile  
 35 40 45

Thr Glu Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
 50 55 60

Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly  
 65 70 75 80

Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Phe Glu  
 85 90 95

Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Ser Trp Asn  
 100 105 110

Ile Arg Leu Glu Arg Asp Cys Phe Ile His Lys Ser Ile Tyr His Gly  
 115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly  
 130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg  
 145 150 155 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser  
 165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Thr Leu Pro  
 180 185 190

Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln  
 195 200 205

Thr Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His  
 210 215 220

Val Asn Pro Leu Lys Val His His His His His His His  
 225 230 235

&lt;210&gt; 54

&lt;211&gt; 311

&lt;212&gt; PRT

&lt;213&gt; Acropora millepora

&lt;400&gt; 54

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr



1	5	10	15
His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val	20	25	30
Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Ile Ser Glu Leu Val Ile	35	40	45
Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser	50	55	60
Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp	65	70	75
Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu	85	90	95
Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn	100	105	110
Ile Arg Leu Glu Gly Asn Cys Phe Ile His Lys Ser Ile Phe His Gly	115	120	125
Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Asp	130	135	140
Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg	145	150	155
Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg	165	170	175
Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro	180	185	190
Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln	195	200	205
Thr Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His	210	215	220
Val Ser Leu Ile Pro Arg Pro Trp Arg Pro Gly Ala Cys Asp Val Gly	225	230	235
Pro Asn Ser Pro Tyr Ser Glu Ser Tyr Tyr Asn Ser Leu Ala Val Val	245	250	255
Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg	260	265	270
Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala	275	280	285
Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp	290	295	300
Thr Arg Pro Val Ala Ala His	305	310	

<210> 55  
 <211> 227  
 <212> PRT  
 <213> Acropora millepora

<400> 55

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr  
 1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
 20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile  
 35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
 50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
 65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
 85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn  
 100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Lys Ser Ile Phe His Gly  
 115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Asp  
 130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg  
 145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg  
 165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro  
 180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln  
 195 200 205

Thr Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His  
 210 215 220

Val Thr Leu  
 225

<210> 56  
 <211> 237  
 <212> PRT  
 <213> Acropora millepora

&lt;400&gt; 56

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr  
 1 5 10 15

Arg Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
 20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile  
 35 40 45

Val Lys Pro Lys Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
 50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
 65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
 85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn  
 100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Asn Ser Ile Phe His Gly  
 115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly  
 130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg  
 145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Tyr His Arg  
 165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Val Lys Pro Val Glu Leu Pro  
 180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln  
 195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His  
 210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His  
 225 230 235

&lt;210&gt; 57

&lt;211&gt; 237

&lt;212&gt; PRT

&lt;213&gt; Acropora millepora

&lt;400&gt; 57

Met Ser His Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr  
 1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val

20 25 30  
 Gly Thr Gly Asn Pro Tyr Glu Gly Ser Gln Met Ser Glu Leu Val Ile  
 35 40 45  
 Thr Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
 50 55 60  
 Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly  
 65 70 75 80  
 Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
 85 90 95  
 Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Ser Trp Asn  
 100 105 110  
 Ile Arg Leu Glu Arg Gly Cys Phe Ile His Lys Ser Ile Tyr His Gly  
 115 120 125  
 Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly  
 130 135 140  
 Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg  
 145 150 155 160  
 Gly Asp Val Thr Gly Phe Leu Met Leu Glu Gly Gly Gly Tyr His Asn  
 165 170 175  
 Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Thr Leu Pro  
 180 185 190  
 Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln  
 195 200 205  
 Thr Ala Lys Gly Phe Thr Ala Lys Leu Glu Glu His Ala Ala Ala His  
 210 215 220  
 Val Asn Pro Leu Lys Val Gln His His His His His His  
 225 230 235

&lt;210&gt; 58

&lt;211&gt; 237

&lt;212&gt; PRT

&lt;213&gt; Acropora millepora

&lt;400&gt; 58

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr  
 1 5 10 15  
 His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
 20 25 30  
 Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile  
 35 40 45  
 Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser

50                      55                      60  
 Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
 65                      70                      75                      80  
 Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
                     85                      90                      95  
 Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn  
                     100                      105                      110  
 Ile Arg Leu Glu Gly Asn Cys Phe Ile His Lys Ser Ile Phe His Gly  
                     115                      120                      125  
 Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Asp  
                     130                      135                      140  
 Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg  
 145                      150                      155                      160  
 Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg  
                     165                      170                      175  
 Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro  
                     180                      185                      190  
 Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln  
                     195                      200                      205  
 Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His  
                     210                      215                      220  
 Val Asn Pro Leu Lys Val Lys His His His His His His  
 225                      230                      235

<210> 59  
 <211> 238  
 <212> PRT  
 <213> Acropora millepora

<400> 59

Met Ala Leu Ser Lys His Gly Leu Thr Lys Asp Met Thr Met Lys Tyr  
 1                      5                      10                      15  
 His Met Glu Gly Ser Val Asp Gly His Lys Phe Val Ile Thr Gly His  
                     20                      25                      30  
 Gly Asn Gly Asn Pro Phe Glu Gly Lys Gln Thr Met Asn Leu Cys Val  
                     35                      40                      45  
 Val Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile Leu Ser Ala Thr  
                     50                      55                      60  
 Phe Asp Tyr Gly Asn Arg Val Phe Thr Glu Tyr Pro Gln Gly Met Val  
 65                      70                      75                      80  
 Asp Phe Phe Lys Asn Ser Cys Pro Ala Gly Tyr Thr Trp His Arg Ser

85

90

95

Leu Leu Phe Glu Asp Gly Ala Val Cys Thr Thr Ser Ala Asp Ile Thr  
100 105 110

Val Ser Val Glu Glu Asn Cys Phe Tyr His Asn Ser Lys Phe His Gly  
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Met Thr Thr Asn  
130 135 140

Trp Glu Pro Ser Cys Glu Lys Ile Ile Pro Val Pro Arg Gln Gly Ile  
145 150 155 160

Leu Lys Gly Asp Ile Ala Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg  
165 170 175

Tyr Arg Cys Gln Phe Asp Thr Ile Tyr Lys Ala Lys Ser Asp Pro Lys  
180 185 190

Glu Met Pro Glu Trp His Phe Ile Gln His Lys Leu Thr Arg Glu Asp  
195 200 205

Arg Ser Asp Ala Lys Asn Gln Lys Trp Gln Leu Val Glu His Ala Val  
210 215 220

Ala Ser Arg Ser Ala Leu Pro Gly His His His His His His  
225 230 235

<210> 60

<211> 227

<212> PRT

<213> Acropora millepora

<400> 60

Met Ser Val Ile Ala Lys Gln Met Thr Tyr Lys Val Tyr Met Ser Gly  
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys  
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Lys Leu Thr Val Thr Lys Gly Gly  
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Cys Gln Tyr Gly  
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Tyr Val Lys  
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu  
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn  
100 105 110

Cys Phe Ile Tyr His Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn

115                      120                      125  
 Gly Pro Val Met Gln Lys Lys Thr Gln Gly Trp Glu Pro Asn Thr Glu  
 130                      135                      140  
 Arg Leu Phe Ala Arg Asp Gly Met Leu Leu Gly Asn Asn Phe Met Ala  
 145                      150                      155                      160  
 Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Thr Thr  
 165                      170                      175  
 Tyr Lys Ala Lys Lys Pro Val Lys Met Pro Gly Tyr His Tyr Val Asp  
 180                      185                      190  
 Arg Lys Leu Asp Val Thr Asn His Asn Lys Asp Tyr Thr Ser Val Glu  
 195                      200                      205  
 Gln Cys Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His  
 210                      215                      220  
 His His His  
 225

<210> 61  
 <211> 237  
 <212> PRT  
 <213> Acropora nobilis

<400> 61

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr  
 1                      5                      10                      15  
 His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
 20                      25                      30  
 Gly Thr Gly Asn Pro Tyr Glu Gly Thr Gln Met Ser Glu Leu Val Ile  
 35                      40                      45  
 Thr Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
 50                      55                      60  
 Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly  
 65                      70                      75                      80  
 Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Cys Glu  
 85                      90                      95  
 Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Ser Trp Asn  
 100                      105                      110  
 Ile Arg Leu Glu Arg Asp Cys Phe Ile His Lys Ser Ile Tyr His Gly  
 115                      120                      125  
 Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly  
 130                      135                      140  
 Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg

145                      150                      155                      160  
 Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Tyr His Ser  
                                  165                      170                      175  
 Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Ala Ala Leu Pro  
                                  180                      185                      190  
 Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln  
                                  195                      200                      205  
 Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His  
                                  210                      215                      220  
 Val Asn Pro Leu Lys Val Gln His His His His His His  
                                  225                      230                      235

<210> 62  
 <211> 237  
 <212> PRT  
 <213> Acropora nobilis

<400> 62

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr  
 1                      5                      10                      15  
 His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val  
                                  20                      25                      30  
 Gly Thr Gly Asn Pro Tyr Glu Gly Thr Gln-Met Ser Glu Leu Val Ile  
                                  35                      40                      45  
 Thr Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
                                  50                      55                      60  
 Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly  
 65                      70                      75                      80  
 Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
                                  85                      90                      95  
 Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Gly Trp Asn  
                                  100                      105                      110  
 Ile Arg Leu Glu Arg Asp Cys Phe Ile His Lys Ser Ile Tyr His Gly  
                                  115                      120                      125  
 Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly  
                                  130                      135                      140  
 Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg  
 145                      150                      155                      160  
 Gly Asp Val Thr Gly Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser  
                                  165                      170                      175  
 Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Ala Ala Leu Pro



180 185 190

Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln  
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His  
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His  
225 230 235

<210> 63  
<211> 237  
<212> PRT  
<213> Acropora nobilis

<400> 63

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Glu Met Lys Thr Lys Tyr  
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Val Glu Gly Val  
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Glu Gln Met Ser Glu Leu Val Ile  
35 40 45

Ile Glu Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser  
50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp  
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu  
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Lys  
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Asn Ser Ile Phe Asn Gly  
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Glu Lys Lys Thr Ile Gly  
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg  
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg  
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro  
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln  
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His

210 215 220

Val Asn Pro Leu Lys Val Lys His His His His His His  
 225 230 235

<210> 64  
 <211> 232  
 <212> PRT  
 <213> *Agaricia fragilis*

<400> 64

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly  
 1 5 10 15

Thr Val Asn Gly His Ala Leu Thr Ile Glu Gly Lys Gly Lys Gly Asp  
 20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala  
 35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly  
 50 55 60

Asn Arg Val Phe Ala Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys  
 65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu  
 85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys  
 100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro  
 115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser  
 130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn  
 145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg  
 165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe  
 180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn  
 195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys  
 210 215 220

Val Ala His His His His His His  
 225 230

<210> 65  
 <211> 306  
 <212> PRT

<213> Agaricia fragilis

<400> 65

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly  
 1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp  
 20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala  
 35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly  
 50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys  
 65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu  
 85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys  
 100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro  
 115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser  
 130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn  
 145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg  
 165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe  
 180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn  
 195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys  
 210 215 220

Val Ala His His His His Ile Thr Asn Lys Ser Arg Gly His Gly Gly  
 225 230 235 240

Arg Glu His Ala Thr Ser Gly Pro Ile Arg Pro Ile Val Ser Arg Ile  
 245 250 255

Thr Ile His Trp Pro Ser Phe Tyr Asn Val Val Thr Gly Lys Thr Leu  
 260 265 270

Ala Leu Pro Asn Leu Ile Ala Leu Gln His Ile Pro Leu Ser Pro Ala  
 275 280 285

Gly Val Ile Ala Lys Arg Pro Ala Pro Ile Ala Leu Pro Asn Ser Cys  
 290 295 300

Ala Ala  
 305

<210> 66  
 <211> 232  
 <212> PRT  
 <213> *Agaricia fragilis*

<400> 66

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly  
 1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp  
 20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala  
 35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly  
 50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys  
 65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu  
 85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys  
 100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro  
 115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser  
 130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn  
 145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg  
 165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe  
 180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn  
 195 200 205

Val Ala Leu Tyr Gly Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys  
 210 215 220

Val Ser His His His His His His  
225 230

<210> 67  
<211> 232  
<212> PRT  
<213> Agaricia fragilis

<400> 67

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly  
1 5 10 15  
Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp  
20 25 30  
Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala  
35 40 45  
Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly  
50 55 60  
Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys  
65 70 75 80  
Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu  
85 90 95  
Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys  
100 105 110  
Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro  
115 120 125  
Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser  
130 135 140  
Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn  
145 150 155 160  
Val Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg  
165 170 175  
Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe  
180 185 190  
Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn  
195 200 205  
Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys  
210 215 220  
Val Ala His His His His His His  
225 230

<210> 68  
<211> 232

&lt;212&gt; PRT

&lt;213&gt; Agaricia fragilis

&lt;400&gt; 68

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly  
1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Glu Gly Asp  
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala  
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly  
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys  
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu  
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys  
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro  
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser  
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn  
145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg  
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe  
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn  
195 200 205

Val Ala Leu Tyr Gly Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys  
210 215 220

Val Ala His His His His His His  
225 230

&lt;210&gt; 69

&lt;211&gt; 232

&lt;212&gt; PRT

&lt;213&gt; Agaricia fragilis

&lt;400&gt; 69

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly

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<210> 70
<211> 232
<212> PRT
<213> Agaricia fragilis
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Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly  
1                   5                   10                   15

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala

35 40 45  
 Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly  
 50 55 60  
 Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys  
 65 70 75 80  
 Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu  
 85 90 95  
 Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys  
 100 105 110  
 Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro  
 115 120 125  
 Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser  
 130 135 140  
 Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn  
 145 150 155 160  
 Thr Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg  
 165 170 175  
 Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe  
 180 185 190  
 Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn  
 195 200 205  
 Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys  
 210 215 220  
 Val Ala His His His His His His  
 225 230

&lt;210&gt; 71

&lt;211&gt; 717

&lt;212&gt; DNA

&lt;213&gt; Acropora aculeus.

&lt;400&gt; 71

atgtcttatt caaagcaggg catcgtaaa gaaatgaaga cgaaataccg tatggaaggc 60  
 agtgtcaatg gccatgaatt cacgatcgaa ggtgtaggaa ctgggtaccc ttacgaaggg 120  
 aaacagatgt ccgaattagt gatcatcaag cctaaggga agccccttcc attctccttt 180  
 gacatactgt catcagtctt tcaatatgga aacagggtgct tcacaaagta ccctgcagac 240  
 atgcctgact atttcaagca agcattccca gatggaatgt catatgaaag gtcattttcta 300  
 tttgaggatg gagcagttgc tacagccagc tggaacattc gtctcgaagg aaattgcttc 360  
 atccacaatt ccattctttca tggcgtaaac tttcccgatg atggaccgt aatgaaaaag 420



aagacaattg gctgggataa gtccttcgaa aaaatgactg tgtctaaaga ggtgttaaga 480  
ggtgatgtga ctatgtttct tatgctcgaa ggaggtggtt accacagatg ccagtttcac 540  
tccacttaca aaacagagaa gccggtcgaa ctgccccga atcatgtcgt agaacatcaa 600  
attgtgagga ccgaccttgg ccaaagtga aaaggcttca cggtaagct ggaagcacat 660  
gctgcggtc atgttaacct tttgaagggt caacagcacc atcaccatca ctaataa 717

<210> 72

<211> 717

<212> DNA

<213> Acropora aculeus

<400> 72

atgtctcttt caaagcatgg catcacacaa gaaatgccga cgaaatacca tatgaaaggc 60  
agtgtcaatg gccatgaatt cgagatcgaa ggtgtaggaa ctggacaccc ttacgaaggg 120  
acacacatgg ccgaattagt gatcataaag cctgcgaggaa aacccttcc attctccttt 180  
gacatactgt caacagtcatt tcaatacggaa aacagatgct tcactaagta ccctgcagac 240  
ctgcctgact atttcaagca agcataccca ggtggaatgt catatgaaag gtcatttgtg 300  
tatcaggatg gaggaattgc tacagcgagc tggaacgtta gtctcgaggg aaattgcttc 360  
atccacaaat ccacctatct tgggtgtaaac tttcctgctg atggaccctt aatgacaaag 420  
aagacaattg gctgggataa agcctttgaa aaaatgactg ggttcaatga ggtgttaaga 480  
ggtgatgtga ctgagtttct tatgctcgaa ggaggtggtt accattcatg ccagtttcac 540  
tccacttaca aaccagagaa gccggtcgaa ctgccccga atcatgtcat agaacatcac 600  
attgtgagga ccgaccttgg caagactgca aaaggcttca tggtaagct ggtacaacat 660  
gctgcggtc atgttaacac tttgaagggt caacatcacc atcaccatca ctaataa 717

<210> 73

<211> 665

<212> DNA

<213> Acropora aculeus

<400> 73

atggaaggca gtgtcaatgg ccatgaattc acgatcgaag gtgtaggaaac tgggtaccct 60  
tacgaaggga agcagatgtc cgaattagt atcgtcaagc ctaagggaac gcccttcca 120  
ttctcctttg acatactgtc atcagttctt caatatggaa acaggtgctt cacaaggtac 180  
cctgcagaca tgctgacta tttcaagcaa gcattcccag atggaatgtc atatgaaagg 240  
tcatttctat ttgaggatgg agcagttgct acagccagct ggaacattcg tctogaagga 300  
aattgcttca tccacaattc catctttcat ggcgtaaact ttcccgtga tggaccctga 360

atgaaaaaga agacaattgg ctgggataag tccttcgaaa aaatgactgt gtctaaagag 420  
 gtgttaagag gtgatgtgac tatgtttctt atgctcgaag gaggtgggta ccacagatgc 480  
 cagtttcact ccacttacaa aacagtgaag ccggtcgaac tgcccccgaa tcatgtcgt 540  
 gaacatcaaa ttgtgaggac cgaccttggc caaagtgcaa aaggcttcac agtcaagctg 600  
 gaagcacatg ctgcggctca tgtaaccctt tgaagggtca acatcaccat caccatcact 660  
 aataa 665

<210> 74  
 <211> 717  
 <212> DNA  
 <213> *Acropora aculeus*

<400> 74  
 atgtctcttt caaagcatgg catcacacaa gaaatgccga cgaaatacca tatgaaaggc 60  
 agtgtcaatg gccatgaatt cgagatcgaa ggtgtaggaa ctggacaccc ttacgaaggg 120  
 acacacatgg ccgaattagt gatcataaag cctgcgggaa aacccttcc attctccttt 180  
 gacatactgt caacagtcac tcaatacgga aacagatgct tactaagta ccctgcagac 240  
 ctgcctgact atttcaagca agcatacca ggtggaatgt catatgaaag gtcatttgta 300  
 tttcaggatg gaggaattgc tacagcgagc tggaacgtcg gtctcgaggg aaattgcttc 360  
 atccacaaat ccacctatct tgggtgtaaac tttcctgctg atggaccctg aatgacaaag 420  
 aagacaattg gctgggataa agcctttgaa aaaatgactg ggttcaatga ggtgttaaga 480  
 ggtgatgtga ctgagtttct tatgctcgaa ggaggtgggt accattcatg ccagtttcac 540  
 tccacttaca aaccagagaa gccggtcaaa ctgccccga atcatgtcat agaacatcac 600  
 attgtgagga ccgaccttgg caagactgca aaaggcttca tgggtcaagct ggtacaacat 660  
 gctgcggctc atgttaaccc tttgaagggt caacatcacc atcaccatca ctaataa 717

<210> 75  
 <211> 717  
 <212> DNA  
 <213> *Acropora aculeus*

<400> 75  
 atgtctcttt caaagcatgg catcacacaa gaaatgccga cgaaatacca tatgaaaggc 60  
 aatgtcaatg gccatgaatt cgagatcgaa ggtgtaggaa ctggacaccc ttacgaaggg 120  
 acacacatgg ccgaattagt gatcataaag cctgcgggaa aacccttcc attctccttt 180  
 gacatactgt caacagtcac tcaatacgga aacagatgct tactaagta ccctgcagac 240

ctgcctgact atttcaagca agcgtaccca ggtggaatgt catatgaaag gtcatttgta 300  
 tttcaggatg gaggaattgc tacagcgagc tggaaacttg gtctcgaggg aaattgcttc 360  
 atccacaaat ccacctatct tgggtgaaac tttcctgctg atggaccctg aatgacaaag 420  
 aagacaattg gctgggataa agcctttgaa aaaatgactg ggttcaatga ggtgttaaga 480  
 ggcgatgtga ctggggttct tatgctcgaa ggaggtggtt accattcatg ccagtttcac 540  
 tccacttaca aaccagagaa gccggtcaaa ctgccccga atcatgtcat agaacatcac 600  
 attgtgagga ccgaccttg caagactgca aaaggcttca tggtaagct ggtacaacat 660  
 gctgcggtc atgtgaaccc tttgaagggt caacatcacc atcaccatca ctaataa 717

<210> 76  
 <211> 666  
 <212> DNA  
 <213> Acropora aculeus

<400> 76  
 atgacctaca aggtttatat gtcaggcacg gtcaatggac attactttga ggtcgaaggc 60  
 gatggaaaag gaaagcctta cgagggggag cagacggtga agctcactgt caccaaggga 120  
 ggacctctgc catttgcttg ggatatttta tcaccacagt cacagtacgg aagcatacca 180  
 ttcaccaa at accctgacga catccctgac tatgtaaagc agtcattccc ggagggatat 240  
 acatgggaga ggatcatgaa ctttgaagat ggtgcagtgt gtactgtcag caatgattcc 300  
 agcatccaag gcaactgttt catctacaat gtcaagttct ctggtttgaa ctttcctccc 360  
 aatggaccgg ttatgcagaa gaagacacag ggctgggaac ccaacactga gcgtctcttt 420  
 gcacgagatg gaatgctgat aggaaacaac tttatggctc tgaagttaga aggaggtggt 480  
 cactatttgt gtgaattcaa atctacttac aaggcaaaga agcctgtgag gatgccaggg 540  
 tatcactatg ttgaccgcaa actggatgta accaatcaca acagggatta cacttccggt 600  
 gaggcgcgtg aaatttccat tgcacgcaaa cctgtggtcg cccatcacca tcaccatcac 660  
 taataa 666

<210> 77  
 <211> 687  
 <212> DNA  
 <213> Acropora aculeus

<400> 77  
 atgagtgtga tcgctaaaca aatgacctac aaggtttata tgtcaggcac ggtcaatgga 60  
 cattactttg aggtcgaagg cgatggaaaa ggaaagcctt acgaggggga gcagacggtg 120  
 aagctcactg tcaccaaggg aggacctctg ccatttgctt gggatatttt atcaccgcag 180

tcacagtacg gaagcatacc attcaccaaa taccctgacg acatccctga ctatgtaaag 240  
 cagtcattcc cggagggata tacatgggag aggatcatga actttgagga tgggtgcagtg 300  
 tgtactgtca gcaatgattc cagcatccaa ggcaactgtt tcactctaaa tgtcaagttc 360  
 tctggtttga actttcctcc caatggaccg gttatgcgga agaagacacg gggctgggaa 420  
 cccaacactg agcgtctctt tgcacgggat ggaatgctga taggaaacaa ctttatggct 480  
 ctgaagttag aaggaggtgg tcactatttg tgtgaattca aatctactta caaggcaaag 540  
 aagcctgtga ggatgccagg gtatcactat gttgaccgca aactggatgt aaccaatcac 600  
 aacagggatt acacttccgt tgagcagtgt gaaatttcca ttgcacgcaa acctgtggtc 660  
 gcccatcacc atcaccatca ctaataa 687

<210> 78  
 <211> 666  
 <212> DNA  
 <213> Acropora hyacinthus

<400> 78  
 atgacctaca aggtttatat gtcaggcacg gtcaatggac actactttga ggtcgaaggc 60  
 gatggaaaag gaaagcctta cgagggggag caaacggtaa ggctgactgt caccaagggc 120  
 ggacctctgc cgtttgcttg ggatatttta tcaccacagt cacagtacgg aagcatacca 180  
 ttcaccaagt accctgaaga catccctgac tatgtgaagc agtcattccc ggagggatat 240  
 acatgggaga ggatcatgaa ctttgaagat ggtgcagtgt gtactgtcag caatgattcc 300  
 agcatccaag gcaactgttt catctaccat gtcaagttct ctggtttgaa ctttcctccc 360  
 aatggacctg ttatgcagaa gaagacacag ggctgggaac ccaacactga gcgtctcttt 420  
 gcacgagatg gagttctgat aggaaacaac tttatggccc tgaagttaga aggaggtgg 480  
 cactatttgt gtgaattcaa atctacttac aaggcaaaga agcctgtgaa gatgcctggg 540  
 tatcactttg ttgaccgcaa actggatgta accaatcaca acaaggatta cacttctgtt 600  
 gagcagcgtg aaatttccat tgcacgcaaa cctgtggctg cccaccacca tcaccatcac 660  
 taataa 666

<210> 79  
 <211> 684  
 <212> DNA  
 <213> Acropora millepora

<400> 79  
 atgaagacga aataccatat ggaaggcagt gtcaatggcc atgaattcac gatcgaagg 60

gtaggaactg gaaaccctta cgaaggcaca cagatgtccg aattagtgat caccgagcct 120  
 gcaggaaaac cccttccatt ctcttttgac attctgtcaa cagtctttca gtatggaaac 180  
 aggtgcttca caaagtaccc tgaaggaatg actgactatt tcaagcaagc attcccagat 240  
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<210> 80  
 <211> 681  
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 <213> *Acropora millepora*

<400> 80  
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 aaacagatat ccgaattagt gatcatcaag cctgcgggaa aacccttcc attctccttt 180  
 gacatactgt catcagtctt tcaatatgga aacaggtgct tcacaaagta ccctgcagac 240  
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 aagacaattg actgggataa gtccttcgaa aaaatgactg tgtctaaaga ggtgctaaga 480  
 ggtgacgtga ctatgtttct tatgctcgaa ggaggtggtt ctcacagatg ccaatttcac 540  
 tccacttaca aaacagagaa gccggtcaca ctgccccga atcatgtcgt agaacatcaa 600  
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<210> 81  
 <211> 684  
 <212> DNA

<213> Acropora millepora

<400> 81

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aaacagatgt ccgaattagt gatcatcaag cctgcgggaa aaccccttcc attctccttt      180
gacatactgt catcagtctt tcaatatgga aacagggtgct tcacaaagta ccctgcagac      240
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atccacaaat ccatctttca tggcgtaaac tttcccgctg atggaccctg aatgaaaaag      420
aagacaattg actgggataa gtccttcgaa aaaatgactg tgtctaaaga ggtgctaaga      480
ggtgacgtga ctatgtttct tatgctcgaa ggaggtgggt ctacagatg ccaatttcac      540
tccacttaca aaacagagaa gccggtcaca ctgccccga atcatgtcgt agaacatcaa      600
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gtgcgggctc atgtaaccct ttga                                             684
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<210> 82

<211> 717

<212> DNA

<213> Acropora millepora

<400> 82

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aagcagatgt ccgaattagt gatcgtcaag cctaaggga agcccttcc attctccttt      180
gacatactgt catcagtctt tcaatatgga aacagggtgct tcacaaagta ccctgcagac      240
atgcctgact atttcaagca agcattccca gatggaatgt catatgaaag gtcatttcta      300
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aagacaattg gctgggataa gtccttcgaa aaaatgactg tgtctaaaga ggtgttaaga      480
ggtgatgtga ctatgtttct tatgctcgaa ggaggtgggt accacagatg ccagtttcac      540
tccacttaca aaacagtga gccggtcgaa ctgccccga atcatgtcgt agaacatcaa      600
attgtgagga ccgaccttgg ccaaagtga aaaggcttca cagtcaagct ggaagcacat      660
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 <212> DNA  
 <213> *Acropora millepora*

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 atgactgact atttcaagca agcattccca gatggaatgt catatgaaag gtcatttcta 300  
 tatgaggatg gaggagtgc tacagccagc tggaacattc gtcttgagag aggttgcttc 360  
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 aagaccattg gctgggataa ggccttcgaa aaaatgactg tgtccaaaga cgtgttaaga 480  
 ggtgatgtga ctgggtttct tatgctcgaa ggagggtggt accacaactg ccagtttcac 540  
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 attgtgagga ctgaccttg ccaaactgca aaagggttca cagccaagct ggaagaacat 660  
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<210> 84  
 <211> 684  
 <212> DNA  
 <213> *Acropora millepora*

<400> 84  
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 gcgggaaaac cccttccatt ctcccttgac atactgtcat cagtctttca atatggaaac 180  
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 cccgctgatg gaccgtaat gaaaaagaag acaattgact gggataagtc cttcgaaaaa 420  
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catcaccatc accatcacta ataa

684

&lt;210&gt; 85

&lt;211&gt; 687

&lt;212&gt; DNA

&lt;213&gt; Acropora millepora

&lt;400&gt; 85

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cctgctgatg gacctgtgat gaaaaagatg acaactaatt gggagccatc ctgcgagaaa 420

atcataccag tacctagaca ggggatattg aaaggggata ttgccatgta cctccttctg 480

aaggatggtg ggcgttatcg gtgccagttc gacacaattt acaaagcaaa gtctgacccg 540

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ggacatcacc atcaccatca ctaataa 687

&lt;210&gt; 86

&lt;211&gt; 666

&lt;212&gt; DNA

&lt;213&gt; Acropora millepora

&lt;400&gt; 86

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gatggaaaag gtaagcccta cgagggggag cagacggtaa agctcactgt caccaagggc 120

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cactatttgt gtgaattcaa aactacttac aaggcaaaga agcctgtgaa gatgccaggg 540

tatcactatg ttgaccgcaa actggatgta accaatcaca acaaggatta cacttcgggt 600



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<210> 87  
<211> 717  
<212> DNA  
<213> *Acropora nobilis*

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tcacttaca aaccagaaaa gccggctgca ctgccccga atcatgtcgt agaacatcac 600  
attgtgagga ctgaccttg ccaagtgc aaaggcttca cagtcaagct ggaagaacat 660  
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<210> 88  
<211> 684  
<212> DNA  
<213> *Acropora nobilis*

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ggtggttacc acagctgcc a gtttcaactcc acttacaaac cagaaaagcc ggctgcactg 540  
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<210> 89  
 <211> 684  
 <212> DNA  
 <213> *Acropora nobilis*

<400> 89  
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<210> 90  
 <211> 681  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 90  
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 aatgttgctt tgtatgaggc agcagttgct cgtcattctc cgctgcctaa ggttgctcat 660  
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<210> 91  
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 <212> DNA  
 <213> *Agaricia fragilis*

<400> 91  
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<210> 92  
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 <212> DNA  
 <213> *Agaricia fragilis*

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<210> 93  
 <211> 678  
 <212> DNA  
 <213> *Agaricia fragilis*

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 catcaccatc actaataa 678

<210> 94  
 <211> 681  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 94  
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<210> 95  
 <211> 681  
 <212> DNA  
 <213> *Agaricia fragilis*

<400> 95  
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<210> 96  
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caccatcacc atcactaata a 681

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(54) Title: NOVEL FLUORESCENT AND COLORED PROTEINS, AND POLYNUCLEOTIDES THAT ENCODE THESE PROTEINS

(57) Abstract:

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International Patent Classification (IPC) or both national classification and IPC		
Applicant UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC.		


This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below

1. ☐ The subject matter of the international application relates to:
  - a. ☐ scientific theories.
  - b. ☐ mathematical theories
  - c. ☐ plant varieties.
  - d. ☐ animal varieties.
  - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
  - f. ☐ schemes, rules or methods of doing business.
  - g. ☐ schemes, rules or methods of performing purely mental acts.
  - h. ☐ schemes, rules or methods of playing games.
  - i. ☐ methods for treatment of the human body by surgery or therapy.
  - j. ☐ methods for treatment of the animal body by surgery or therapy.
  - k. ☐ diagnostic methods practised on the human or animal body.
  - l. ☐ mere presentations of information.
  - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
 

☐ the description
 ☐ the claims
 ☐ the drawings
3. ☒ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out:
 

☐ the written form has not been furnished or does not comply with the standard.
 ☒ the computer readable form has not been furnished or does not comply with the standard.
4. ☐ The failure of the tables related to the nucleotide and/or amino acid sequence listing to comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions prevents a meaningful search from being carried out:
 

☐ the written form has not been furnished.
 ☐ the computer readable form has not been furnished or does not comply with the technical requirements.
5. Further comments: The requested Sequence Listing on floppy was not furnished

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  Henriëtte Huysing-Solles
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## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 203

The claims of the underlying application have, according to PCT Rule 13ter.1.c, not been searched since the Sequence Listing has not been furnished in machine readable form as prescribed in the administrative instructions under Rule 5:2. The applicant has not remedied the disclosed deficiencies within the time limit fixed in the invitation pursuant to PCT Rule 13ter.1.a.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO, (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

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